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Sir Archibald Garrod's "Inborn Errors of Metabolism"

II. Alkaptonuria

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"Of inborn errors of metabolism, alkaptonuria is that of which we know most, and from the study of which most has been learnt." *Alkaptonuria*, A. E. Garrod, 1908.

INTRODUCTION

THE LINES ABOVE, quoted from the opening of Garrod's Croonian Lecture on Alkaptonuria, are as true today as when written, though this fact cannot be easily confirmed by casual reading of the available texts. The usual source presents only a restricted view of what has been a rich and many-faceted problem, and one which in turn is still enriching our understanding of biological processes. This disease was the prototype of the inborn errors of metabolism. It was the first hereditary human disease whose mode of transmission was known, and through its use the first intermediary metabolic pathway was elucidated. The papers marking the milestones in its study constitute an interdisciplinary education in clinical chemistry (Bödeker, 1861), pathology (Virchow, 1866), organic chemistry (Wolkow and Baumann, 1891), internal medicine (Osler, 1904), biochemistry (Neubauer, 1928), statistical genetics (Hogben, Worrall & Zieve, 1932), plus recent work too near to characterize, and above all a conceptual scheme on a grand scale which cannot be otherwise characterized (Garrod, 1902). It is intended here to trace the history of our knowledge of alkaptonuria and critically to evaluate the advances that have been made in its study in the fifty years since Garrod's lectures introduced it as the type example of a new class of disease.

Alkaptonuria consists of the life-long excretion in the urine, after the first few days of life, of the strongly reducing substance, homogentisic acid. This is a normal intermediary metabolite formed in the body from tyrosine. It accumulates because the enzyme reaction normally oxidizing it is missing. Homogentisic acid can be spontaneously oxidized in alkaline medium to a brown or black polymer. This polymer causes the most famous signs of the disease: old urine is darkened and wetted clothes are stained. Something like this same black polymer also accumulates slowly in the body in certain mesenchymal tissues, producing by middle life the blackening of

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cartilages and related tissues called ochronosis. This coloration can often be observed through the skin, but when seen in the dissected tissues is a most arresting sight. At least some of the dyed tissues degenerate prematurely. Arthritis, the only result of this degeneration now generally recognized, is almost inevitable in this disease after middle age and it may be incapacitating.

The vast majority of the cases of alkaptonuria are adequately accounted for on the basis of a single autosomal recessive gene hypothesis. A few pedigrees showing direct transmission of alkaptonuria from one generation to the next have given rise to the suggestion that there may also be a very, very rare, dominant form. The evidence for the existence of a dominant form is weak, and it is more likely that all cases conform to the recessive mode of inheritance.

I. HISTORY

Sir Archibald Garrod was, among other things, a scholar with a beautiful and lucid style which makes him one of the most quotable of medical writers. His version of the history of alkaptonuria deserves to be given in his own words, and especially so since it has been the source of the introductory paragraphs of so many papers about this disease: "Until the early years of the nineteenth century no distinction was drawn in medical writings between urines which were black when passed and such as darkened on exposure to air, but it is difficult to suggest any other diagnosis than that of alkaptonuria for some cases referred to in works of the sixteenth and seventeenth centuries, such as that mentioned by G. A. Scribonius (in 1584) of a schoolboy who, although he enjoyed good health, continuously excreted black urine, and that cited by Schenck (in 1609) of a monk who exhibited a similar peculiarity and stated that he had done so all his life. The most interesting record of this kind is to be found in the work of Zacutus Lusitanus, published in 1649. The patient was a boy who passed black urine and who, at the age of fourteen years, was submitted to a drastic course of treatment which had for its aim the subduing of the fiery heat of his viscera, which was supposed to bring about the condition in question by charring and blackening his bile. Among the measures prescribed were bleedings, purgation, baths, a cold and watery diet, and drugs galore. None of these had any obvious effect, and eventually the patient, who tired of the futile and superfluous therapy, resolved to let things take their natural course. None of the predicted evils ensued, he married, begat a large family, and lived a long and healthy life, always passing urine black as ink." (Garrod, 1908).

It would appear that undue emphasis has been placed upon the darkening of alkaptonuric urine, since many know of it only in this way. Black urine, of course, is one of the more striking of the manifestations of this whole group of diseases "which advertise their presence in some conspicuous way, either by some strikingly unusual appearance of surface tissues or of excreta, by the excretion of some substance which responds to a test habitually applied in the routine of clinical work, or by giving rise to obvious morbid symptoms." (Garrod, 1909, p. 16). But the urine blackens slowly by itself, and must be looked at a day later with seeing eyes to note the change, while its response "to a test habitually applied in the routine of clinical work" is much more regularly observed by the busy practicioner. This test, one for the reducing action of

urine to detect diabetes, is responsible for most of the diagnoses of alkaptonuria. It is also the reason why most of the early patients, and about half of those seen even today, were considered for a shorter or longer time to be diabetics.

The First Patient: The confusion of diabetes and alkaptonuria was to be expected when Bödeker (1859, 1861) described the first alkaptonuric patient seen in modern times as also having glycosuria. We can now distinguish glucose by the specific tests for it. Alkaptonuric urine can be differentiated by its negative results with bismuth reduction, fermentation, phenylhydrazine precipitation and optical rotation, and identified by its blackening of undeveloped photographic film (Fishberg, 1942). Yet when Bödeker first met this problem he fared better than many of his successors. The urine reduced Fehling's solution as if it contained glucose, but it did not reduce bismuth hydroxide (e. g., the Nylander reaction), a test still useful in distinguishing homogentisic acid from glucose. From the result of these tests and others, he concluded that the reduction was not caused by sugar. Yet the bias that reducing urine meant diabetes was strong. By greatly concentrating the urine, he detected some fermentation with yeast, and concluded that sugar was present "above the usual small quantities—certainly not above 1 per cẽnt." From his amazement that the patient continued well, without polyuria or other diabetic symptoms, it is clear that he thought he was dealing with an atypical form of diabetes.

What had first caught Bödeker's attention was the color change of the urine as he made it alkaline for the test for sugar. This observation was made in the course of a routine examination of urine from a forty-four year old man, hospitalized without relief, it should be noted, from his incapacitating arthritic pain in the lumbar spine. "Sobald ich Behufs der Prüfung auf Zucker den Harn zuerst nur mit etwas Aetznatron kalt mischte, sah ich, sie die blass röthlichgelbe Flüssigkeit sich von oben herab auffallend braun verdunkelte. . . . " As the urine darkened *from the surface*, "it took up somewhat more than its own volume of oxygen gas," and this gave the substance its name. "I call it for this reason 'Alkapton' (admittedly a somewhat barbarous combination from the Greek participle of *κάπτειν*, to suck up greedily, and the Arabic *al kali*), after its outstanding behaviour toward oxygen in alkaline solution." It should be mentioned here that the word, alkaptonuria, is not less of a barbarism when the "k," which is at least half Arabic in origin, is changed to "c" as is frequently done in the English-speaking world.

Bödeker isolated by lead precipitation and ether extraction, methods which became classical, a sugar-free but nitrogen-contaminated, crystalline sample of his "alkapton." Before he could obtain more for determination of its structure, the patient left the hospital. Bödeker's "fervent hope" that the man would again seek hospitalization was after two years still "sadly unfulfilled," although he then received "a couple of ounces of urine," enough to repeat the qualitative tests and establish the condition as a chronic one. Not for thirty years was another of these rare patients to come into the hands of a biochemist equally as competent as Bödeker. Although he did not find the structure of the "alkapton," he had recognized that its uptake of oxygen in alkaline solution with the formation of dark brown or black pigment was similar to the behavior of hydroxyphenols like pyrogallol and quinone.

The Search for "Alkapton": The subsequent guesses about the chemical nature of

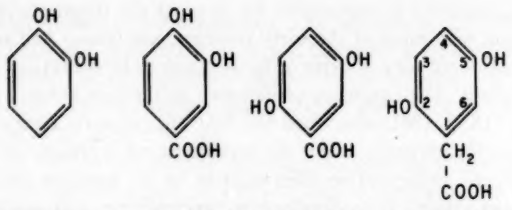


FIG. 1. Structural formulae suggested for the "alkapton", and of the metabolite of salicylic acid, gentisic acid, from which homogentisic acid derived its name.

the compound kept closely to the hydroxyphenols with which Bödeker compared it, but the names given it were limited only by the number of cases seen before 1891, when the structure was at last established. Not until 1875 was alkaptonuria again reported, and then two cases were found. Ebstein and Muller (1875) assumed that the substance excreted was pyrocatechol, simply on the basis of its color with ferric chloride. For this reason their patient was excluded from the series Garrod later collected, although the urine tests were typical of alkaptonuria. Fleischer (1875) then obligingly isolated a small amount of pyrocatechol (Fig. 1) from the urine of Fürbringer's (1875) patient, and though he also isolated similar amounts from the urines of several non-alkaptonurics, the German workers (including Bödeker—see note in Fürbringer, 1875), were in temporary agreement that alkaptonuria was "brenzcatechinuria."

Then cases began to be found abroad. From Dublin, Smith and Armstrong (1882) reported a three year old girl with alkaptonuria. They noted a simple fact, that the "alkapton" could not be extracted into ether unless it was first acidified. This showed that it had an acid group, and they guessed that it was protocatechuic acid. Brune (1886, 1887) proved that the material isolated from the urine of the first American case was not protocatechuric acid. He also stated that all the contemporaneous workers were dealing with the same substance—one which was of no pathological importance since it did not indicate diabetes! The purest compound, with nearly the correct melting point, was isolated by Marshall (1887) from urine of a patient he did not see, one whose "diabetes" prevented his buying life insurance. Marshall called this substance "glycosuric acid" and strangely enough made no mention of its darkening reaction. This patient was actually the same as Brune's (see King, 1915), and was later described by Osler (1904). The patient was the younger of the two brothers in whom ochronosis was first diagnosed clinically, as well as the somewhat overdiagnosed first American case.

Up to this time there had been a remarkable unanimity, despite the several names used, about the properties of the "alkapton" and, most importantly, about the fact that only one substance caused the phenomena observed in the affected urines. This was changed by Kirk in Britain. A charitable assessment of his studies should emphasize that he established the familial incidence of alkaptonuria. Three younger boys in a family had alkaptonuria, while an older boy and the parents were normal.

This important observation was reinforced by Kirk's parenthetic remark (1886) that Smith had written him to say that the three year old Dublin patient now had a sibling, also alkaptonuric. Perhaps mention should also be made of Kirk's belief that the "alkapton" was a disinfectant. He believed its presence in these children accounted for the mildness of their illnesses during a typhoid epidemic when many people on their street were dying (Kirk, 1889a). He could not explain the fact that the youngest child nevertheless died of whooping cough.

By his own admission, chemical matters were beyond Kirk (1889a). He isolated fractions from urine containing the "alkapton" and named them by their colors: "urrhodinic acid" (1886), "uroxanthic acid" (1888) and "uroleucic acid" (1889b). Huppert of Prague may be really to blame for taking seriously this example of phenomenology. He was in search of the latest facts for a new textbook on urine, and though he realized (correspondence in Kirk, 1889a) that all of Kirk's fractions were impure "uroleucic acid," he assumed that at least it was real and different from the "alkapton." Eventually he published a probable structure, based on analyses of Kirk's impure samples (Huppert, 1897), as 2,5-dihydroxyphenyllactic acid. Not until years later was this error corrected, when Garrod saw that the excretion of two substances instead of one would weaken the case for the single metabolic block he envisaged, and when no other patient had been found to excrete more than the one substance homogentisic acid. Garrod then reexamined Kirk's patient. This youth excreted only homogentisic acid (Garrod, 1902). Still later, after Kirk's death, Garrod examined the samples of "uroleucic acid" and found nothing but impure homogentisic acid (Garrod, 1909).

"The New Era": These early experiments were authoritatively reviewed in 1891 by Wolkow and Baumann. They did this with a fine sense of history, for as they said in the introduction to their paper, having now "prepared the alkapton in pure form, determined its structure, shown how it is related to metabolism, and found some of the conditions for its creation . . . the alkapton problem moves into a new era [neues Stadium]." Much of this classical paper is indeed relevant to the next phase of alkaptonuria investigation, the metabolic studies, where it will be discussed. For the moment, it is enough that they furnished elaborate chemical proof that the substance isolated was not one of the sixteen known acids with the same empirical formula, and identified it as a compound unknown till then, 2,5-dihydroxyphenylacetic acid. They named it homogentisic acid, since it was the next higher homologue of the familiar salicylic acid metabolite, gentisic acid. They also carefully outlined the great similarities of the urine from their patient to the urines already described. They left no doubt that where verifiable properties of the isolated substances had been recorded, namely for Marshall's "glycosuric acid" and Brune's isolated compound, these substances were also homogentisic acid. As for Kirk's "uroleucic acid," their first deep red extract, the subsequent yellow fraction, and the final white material seemed very similar, but in the end they proved that such a substance as Kirk had described was entirely absent from the urine they studied. This otherwise excellent work also contained a forceful statement that homogentisic acid was formed by the putrefaction of protein in the gut and not by the tissue metabolism.

An Inborn Error of Metabolism: The chronology of discoveries about alkaptonuria

—the chemical identification of homogentisic acid in 1891—the pathological identification of ochronosis with alkaptonuria in 1902—the clinical recognition of the accompanying arthritis in 1907—the realization about the same time that it was a metabolic disease—would seem to have set the stage for Garrod's grand synthesis in 1908 of the clinical signs with heredity and with step-wise metabolic aberrations. Nothing would be farther from the truth. The concept of alkaptonuria as an inborn error of metabolism was developed almost independently of the above discoveries, and it was formed by 1902 (Garrod, 1902).

There can be no doubt that alkaptonuria was the prototype of the inborn errors of metabolism. It was the first of these diseases that Garrod studied, and the one to which he personally made important contributions. It is also the disease which first fitted and which most closely fits, then and now, the definition he gave for the group. His publications on the subject permit us to follow the genesis of his concept.

Garrod began his studies with the description of five new cases of alkaptonuria (making a total of twenty-eight), almost certainly more than any one person had seen up to that time (Garrod, 1899). His cases confirmed the fact, first reported by Kirk, that this very rare disease might be met in several members of one family. His subsequent contributions were curiously off the main paths of the then current investigations of alkaptonuria. He seemed almost indifferent to much of the reported work. He assembled new information, and verified or disproved earlier statements, but only of a sort which interested him. He was from his first paper a man with an idea.

The sort of information which interested him and was reported in his second paper (Garrod, 1901) concerned the duration, the metabolic nature and the familial incidence of alkaptonuria. The staining tendency of the urine of a new-born child, whose older sibling had already been diagnosed as an alkaptonuric, was described from birth, diaper by diaper. There was slight staining of the diaper thirty-eight hours after birth, deep staining of one after fifty-two hours, and staining of all subsequent ones. Garrod associated the beginning of the alkaptonuria with the child's first feeding. The lag of a day or two before stained napkins were noted, also supported by the history of several additional cases, might now be ascribed to the time necessary for maturation of the phenylalanine and tyrosine oxidizing systems in the liver of a new-born child (Kretchmer *et al.*, 1956; Kenney *et al.*, 1958). Although patients with intermittent alkaptonuria had been described, Garrod was not convinced they occurred (Garrod, 1902), and by the above observations he had demonstrated that the condition was congenital in at least some cases.

Garrod doubted the theory almost forced on the world by Baumann that alkaptonuria resulted from a specific form of infection of the alimentary canal. To check this, he measured the urinary homogentisic acid of his four year old patient at intervals after feeding. He found that the maximal excretion occurred four to seven hours after a meal, coincident with the peak excretion of nitrogen, and not earlier as might have been expected if homogentisic were formed in the gut before the absorption of tyrosine. From this he concluded that tyrosine from the diet must be absorbed and then converted by the body's metabolism to homogentisic acid. "The facts lend support to the view that alkaptonuria is what may be described as a 'freak' of metab-

olism, a chemical abnormality more or less analogous to structural malformations." This is a much more pregnant statement than an earlier one by Kirk (1886), that the anomaly "must result from a profound perversion or arrest of metabolism."

Two new observations about the familial incidence of alkaptonuria reinforced the earliest statement of the concept of inherited biochemical abnormalities. "There is, as yet, no known instance of its transmission from one generation to another. . . ." And later in the same paper, "I am able to bring forward evidence which seems to point, in no uncertain manner, to a very special liability of alkaptonuria to occur in the children of first cousins." The latter information concerned four sibships, all from first cousin marriages, "including no less than eleven alkaptonuric members, or more than a quarter of the recorded examples of the condition" (Garrod, 1901).

The words "freak" and "sport" were used to describe the appearance of alkaptonuria among sibs whose parents were unaffected. It was the language of the day for such phenomena. Darwin had used the same terms for the differences within a species on which selection might act to produce evolutionary changes. Understanding of these matters had not yet gone beyond that possessed by an observant husbandryman, but Garrod had marshalled the important facts of heredity, metabolism and disease. The spark that would fuse them together was then in press: the rediscovery of Mendel's work.

The year 1902 saw the emergence of modern ideas of hereditary disease in a surprisingly mature form. William Bateson published "Mendel's Principles of Heredity" (Bateson, 1902), which contained an explanation of why the mating of first cousins would enable a rare recessive condition to show itself. In an obscure footnote, Bateson actually said that a rare recessive factor would explain the observed incidence of alkaptonuria (Bateson & Saunders, 1902). In the same year Garrod made use of this information and more that he had gathered to publish a précis of the inborn errors of metabolism (Garrod, 1902). The intellectual climate of the times can be gauged from some of its words: "The question of the liability of children of consanguineous marriages to exhibit certain abnormalities or to develop certain diseases has been much discussed, but seldom in a strictly scientific spirit. Those who have written on the subject have too often aimed at demonstrating the deleterious results of such unions on the one hand, or their harmlessness on the other, questions which do not here concern us at all. There is no reason to suppose that mere consanguinity of parents can originate such a condition as alkaptonuria in their offspring, and we must rather seek an explanation in some peculiarity of the parents, which may remain latent for generations, but which has the best chance of asserting itself in the offspring of the union of two members of a family in which it is transmitted" (Garrod, 1902).

Since his last paper on the subject, Garrod had corresponded with many who had investigated alkaptonuria to obtain information they had neglected to publish about the families of these patients. The results were still fragmentary, but "more cannot be learned until new cases are described." For contemporary readers the clarity of the presentation must have suffered, because Garrod sought to do much more than support the recessive hereditary nature of the condition. This paper on "The Incidence of Alkaptonuria: A Study in Chemical Individuality," which Hogben *et al.*,

(1932) called "a landmark in the history of human genetics," is also the key reference for the development of the concept of inborn errors of metabolism.

Garrod (1902) first recorded the results of his reexamination of Kirk's patient, who now excreted only homogentisic acid and no "uroleucic acid." It is not reading too much into this introduction to assume that he checked the finding because he felt the excretion of a single compound, instead of two or more, was the more likely result of a single metabolic block of the sort he had in mind. Next he reviewed his earlier conclusions that alkaptonuria "is not a manifestation of a disease, but is rather of the nature of an alternative course of metabolism, harmless and usually congenital and lifelong" (though as a course of metabolism it was "somewhat inferior to the ordinary plan . . . ," with "a certain slight waste of potential energy."). Next followed a table giving the amounts of homogentisic acid (2.6 to 5.9 g.) excreted per twenty-four hours by nine different patients. None was found in the urine of normal individuals, so the excretion represented a qualitative trait, not simply a quantitative difference. Garrod had learned about the sibships of nineteen patients. These nineteen plus their twenty-nine sibs made up nine families. In one family, that of the two brothers described by Osler, a son also had alkaptonuria, but in all other families only the members of one generation were affected. In ten families about which information on the relationship of the parents was available, six (containing twelve alkaptonurics among thirty-six sibs) were the offspring of first cousin marriages. The parents of four sibships (containing seven alkaptonurics among more than fifteen sibs) were not known to be related. Garrod contrasted this incidence of 60 per cent consanguineous marriages in alkaptonuric families with the estimate by Darwin that less than three per cent of the marriages in England were consanguineous. He concluded that a very rare "latent peculiarity" in the parents asserted itself under these conditions. He was quick to state that this did not occur in most consanguineous marriages, else there would be upwards of 50,000 alkaptonurics in London alone, in place of the mere six he had located by searches at two active hospitals. At the end of his paper Garrod quickly sketched the salient characteristics of a "sport" such as alkaptonuria: a conspicuous and specific chemical deviation occurring among brothers and sisters, often the product of first cousin marriages. Albinism, and possibly cystinuria, might also represent similar conditions, and this would make the first more believable. If so, a new class of diseases would be known.

In the Croonian Lectures, and his first edition of them, Garrod (1908, 1909) noted some evidence in the larger sibships of a 3:1 Mendelian segregation, but he did not further develop the study of the heredity of alkaptonuria. He had become occupied with albinism, cystinuria and a fourth condition, pentosuria, as additional examples which bolstered the validity of his concept. Most of his discussion of alkaptonuria dealt with the biochemical aspects which were then very imperfectly known. In effect, he left those parts of the problem, like the fact of inheritance which was moderately well-established, and turned to work on those aspects which were still without form. Though in his lectures his discussion of the biochemical problems of homogentisic acid excretion was not particularly well-founded on the most recent work, his contribution was of permanent value. He intuitively imposed the correct form on the incomplete and contradictory data available at a time when it was not

really known that metabolism occurred in discrete enzyme-catalyzed steps, when homogentisic acid was widely believed to arise from putrefaction in the gut, and when other evidence identified it as an abnormal compound formed only by alkaptonuric patients. Subsequent work has fully borne out his view of compartmentalized metabolism, each step of which was under hereditary control. The initial statement of this view was the guide to the understanding of alkaptonuria, the basis of his concept of the inborn errors of metabolism, and represented the birth of biochemical genetics: "The conception of metabolism in block is giving place to that of metabolism in compartments. The view is daily gaining ground that each successive step in the building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose.

"It may well be that the intermediate products formed at the several stages have only momentary existence as such, being subjected to further change almost as soon as they are formed; and that the course of metabolism along any particular path should be pictured as in continuous movement rather than as series of distinct steps. If any one step in the process fail the intermediate product in being at the point of arrest will escape further change, just as when the film of a biograph is brought to a standstill the moving figures are left foot in air. All that is known of the course of catabolism tends to show that in such circumstances the intermediate product in being is wont to be excreted as such, rather than that it is further dealt with along abnormal lines. Indeed, it is an arguable question whether, under abnormal conditions, the metabolic processes are ever thrown out of their ordinary lines into entirely fresh paths, with the result that products are formed which have no place in the normal body chemistry" (Garrod, 1908).

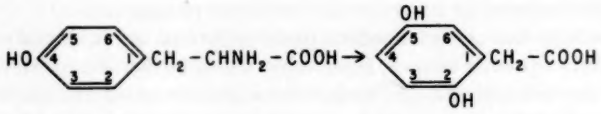
II. METABOLISM

Enough was earlier said of Wolkow and Baumann's (1891) work to identify it as the second milestone in the study of alkaptonuria, to be compared only with Bödeker's (1859) discovery and Garrod's conceptual synthesis (1902). This single paper contained not only a discerning assessment of the virtues and defects of earlier work, and the chemical isolation and identification of the "alkapton" as homogentisic acid, but it also laid the groundwork for the studies that were to occupy biochemistry in this area for the next sixty years. It also contained a very strong statement about the nature of the disease which seems ludicrous today.

As soon as the aromatic ring structure of homogentisic acid was certain, its origin was deduced. Baumann, who had much experience in the study of the origin of excreted compounds, stated flatly that only plants and not animals could make aromatic compounds out of substances without benzene rings. The aromatic compounds such as homogentisic acid that were excreted by animals could come only from the protein in the body or in the diet. There were regularly only two (then) known aromatic substances in protein, tyrosine and phenylalanine. Wolkow and Baumann had in the laboratory sufficiently large samples for metabolic studies only of one of these substances, tyrosine. They developed a method for the quantitative assay of homogentisic acid in urine by silver titration, and fed their patient the tyrosine. The results

TABLE I. ORIGIN OF HOMOGENTISIC ACID FROM TYROSINE OF DIETARY PROTEIN (WOLKOW & BAUMANN, 1891)

Regimen	Homogentisic Acid Excreted (g/day)	
	Total	Extra
Hospital diet (av. 14 days)	4.6	—
Hospital diet + 10 g. Tyrosine	11.5	6.9
Hospital diet + 11.5 g. Tyrosine	14.2	9.4
Meat diet (av. 17 days)	6.4	—
Meat diet + 12.5 g. Tyrosine	15.8	9.4



Tyrosine Homogentisic Acid

FIG. 2. The over-all conversion of tyrosine to homogentisic acid, deemed impossible for mammals in 1891.

showed that the amount of homogentisic acid excreted by their patient was increased with a high protein diet and greatly increased when the tyrosine was fed (table I).

Man or Microorganism? Baumann's chemical knowledge misled him only when he attempted to deduce the mechanism by which the precursor tyrosine was converted to homogentisic acid. The conversion involved the unheard of change of a 4-hydroxyphenyl compound to a 2,5-dihydroxyphenyl compound; i.e., the removal of one hydroxyl group and the appearance of two new ones (Fig. 2).

"Man hat niemals das Verschwinden einer Phenolhydroxylgruppe durch Reduction in den Organen des Thierkörpers beobachtet." In fact, Baumann made an eloquent appeal that if such a conversion occurred in the tissues *which was not already known to chemistry*, the certainty built up by the study of the metabolism of hundreds of compounds over the past decade would be lost. Metabolism might then do anything and to investigate the metabolism of any substance would be useless. He concluded with the observation that, although some excreted substances were intermediate products of metabolism, "Das ist aber bei der Homogentisinsaure . . . ganz und gar nicht der Fall." Having denied the ability for this conversion to man, he had no recourse but to attribute it to the little animals, the bacteria in the gut: "Auf Grund obiger Darlegungen sind wir zu dem Schlusse gelangt, dass die Bildung der Homogentisinsäure aus dem Tyrosin nicht curch eine an sich unerklarbare abnorme Function des Stoffwechsels in den Geweben bedingt, sondern als eine Wirkung einer besonderen Art von Mikroorganismen anzusehen sei." Kluyver and Zijp (1951) greatly enjoyed this quotation while pointing out that in the intervening sixty years no one had been able to find microorganisms that produced homogentisic acid. They, and Utkin (1950), recorded the only known examples, the conversion of tyrosine and phenylacetic acid to homogentisic acid by *Aspergillus niger*.

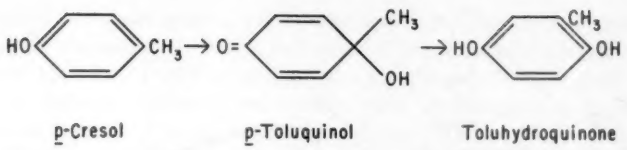


FIG. 3. The chemical reaction of side-chain (CH_3) migration, through an intermediary quinol This reaction served as the prototype of homogentisic acid formation.

The proof offered for this strongly held theory, that the bacteria in the intestine formed homogentisic acid, came from a single attempt at intestinal disinfection. The patient was given 6 g. of salol (phenylsalicylate) daily for three days while the diet was kept constant. The amount of homogentisic acid excreted daily was unchanged, except on the third day when 60 per cent of the usual amount was excreted. Contrary to their usual standards of work, this crucial experiment was not repeated because the patient left the clinic. But the theory was indirectly disproved by a still more important experiment whose meaning was not then fully appreciated. A small dog metabolized nearly all of a dose of 4.5 g. of homogentisic acid. When it was later shown in a similar experiment that a normal man could also metabolize large doses of homogentisic acid (Embden, 1893), it should have been clear that the metabolism was abnormal in the alkaptonuric patient.

Despite the effective work of Baumann's student, Embden, who disproved his master's theory in several ways, Baumann's putrefaction theory of the origin of abnormal metabolites was not weakened until the scientific reason for its proposal was removed. An alternative to the metabolic formation of homogentisic acid was needed only because that type of a chemical transformation was unknown. Meyer (1901) suggested that the side chain instead of the hydroxyl group could migrate on the ring. Soon after, Bamberger (1903) demonstrated the oxidation of *p*-cresol with side chain migration to form toluhydroquinone (Fig. 3). It was seen that this chemical reaction provided an analogy for the reaction of tyrosine to homogentisic acid in metabolism (Friedmann, 1908) and Baumann's theory collapsed.

The many observations on alkaptonuria made in the two decades at the turn of the century could then be ascribed to metabolic processes, but the study was immediately hung on a new dilemma: Did homogentisic acid represent an accumulation of a metabolite before a blocked step of normal metabolism, or was it an abnormal compound formed by an abnormal series of reactions? Whether normal or abnormal, the series of reactions leading to homogentisic acid was determined. Substances that were precursors of homogentisic acid could be distinguished by their causing an increased excretion of homogentisic acid when fed to alkaptonurics. Protein and phenylalanine, as well as tyrosine and homogentisic acid itself, increased the excretion of homogentisic acid in alkaptonuric patients. So did a number of postulated intermediates, while a much larger number of possible intermediates did not increase homogentisic acid excretion, and they were excluded from the projected pathway. This exciting period was later reviewed in detail by one of the main participants (Neubauer, 1928), and Fig. 4, taken from this review, shows the pathway finally deduced. All of the compounds shown, except 2.5-dihydroxyphenylalanine, had been

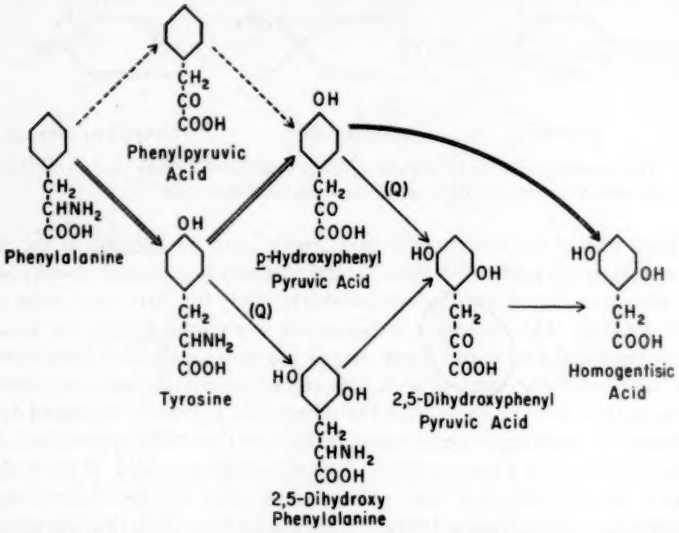


FIG. 4. Possible pathways of the metabolism of phenylalanine to homogentisic acid, deduced from feeding experiments with alkaptonuric patients (Neubauer, 1928). Neubauer indicated the less likely paths with dotted lines. The reactions involving the hypothetical quinol intermediate are marked "Q". The doubled arrows have been added to show the actual pathway (see Fig. 5).

tested. Later Neuberger *et al.*, (1947) synthesized 2,5-dihydroxyphenylalanine and proved that it too was converted by the alkaptonuric patient to homogentisic acid. Neubauer was aware that his method of testing would include compounds which, though not on the direct pathway from phenylalanine to homogentisic acid, would feed freely into the pathway. The final decision about which of the possible routes deduced represented the direct pathway actually used had to await the step by step analysis of the various enzyme reactions in the liver (Knox, 1955).

In the meantime, the objection gained momentum that the first metabolic pathway of some complexity to be elucidated was an abnormal one. To this crucial question Garrod had given a succinct answer: "It appears to me that at present the evidence in favour of the theory of an intermediate product far outweighs that which can be brought against it. Perhaps the most serious objection which can be raised to the view that homogentisic acid is an abnormal product, peculiar to alkaptonurics, is that such a view involves the assumption that the alkaptonuric, who alone has the power of forming homogentisic acid, is also exceptional in having no power of destroying it when formed" (Garrod, 1909). This answer would carry more weight today than it did then, when sequential metabolic reactions were almost unknown and what is now called biochemical genetics was on trial. Dakin, who was sceptical that a normal metabolic pathway had been elucidated, devised an ingenious test of the whole scheme. The over-all reaction was thought to depend upon the intermediate formation of hypothetical quinol, through which a 4-hydroxyphenyl could be converted to a 2,5-dihydroxyphenyl compound (see Fig. 3). A compound blocked in the para

position so it could not form a quinol, like *p*-methyl- or *p*-methoxyphenylalanine, should not be oxidized by a normal individual, if the postulated scheme were indeed the normal metabolic pathway. But when such compounds were given to normal animals, or perfused through liver, or even when administered to an alkaptonuric subject, they were said to be completely oxidized (Dakin, 1911). The conclusion appeared inescapable that the metabolism of these compounds occurred by another route, perhaps the truly normal route and one which did not involve either a quinol or homogentisic acid.

The results of Dakin, confirmed by similar studies (Fromherz & Hermanns, 1914), created a paradox unresolved until recently. Such was the appeal of Garrod's thesis, including the belief in a blocked normal metabolism, that investigations along this line persisted until the metabolic pathway of phenylalanine through homogentisic acid was finally established as the normal and major route. Only recently were the experiments of Dakin repeated. Ichihara (1957) and Pirrung, Gottesman and Crandall (1957) then found that the compounds were not metabolized. It must be inferred that the methods used by the earlier workers could not detect the unchanged compounds that they administered.

There remained some other evidence, which has not yet been explained, against the now accepted normal pathway of phenylalanine metabolism. One example out of many will be given. A three year old boy with alkaptonuria, when fed a diet sufficiently low in carbohydrate to cause ketosis, ceased to excrete homogentisic acid (Katsch, 1918). It appeared that either the blocked reaction began to function in acidosis or the "abnormal" route through homogentisic acid was depressed by ketosis relative to another, perhaps the normal, metabolic route. It was suggested that ketosis decreased the ability of a normal individual to metabolize homogentisic acid (Katsch, 1920). This pointed to the existence of an alternative metabolic route for tyrosine. The careful experiments of Katsch were not confirmed in two adults (Lieb & Lanyar, 1930; Diaz, Mendosa & Rodriguez, 1939). However, the results of Katsch, the examples of intermittent alkaptonuria, and the failures of normal individuals fully to metabolize homogentisic acid "under certain poorly understood circumstances" (see Galdston, Steele and Dobriner, 1952, references 31–49, for additional examples) cannot be overlooked, even if they are not accepted as evidence against Garrod's concept.

The problem may be solved eventually by an understanding of the regulation of metabolism. The metabolic reactions of the body are not simply present and working at full speed in the normal individual, or absent in the individual with a condition like alkaptonuria. Each reaction is modulated to fit the momentary demands of the body and the environment. The enzyme for the first of the reactions in the metabolism of tyrosine is now known to increase ten-fold in amount in a few hours after tyrosine administration, or after hydrocortisone treatment (Lin & Knox, 1957a). These instances of metabolic adaptation (Knox, Auerbach & Lin, 1956) are a sort of temporary somatic variation superimposed on the genetically determined metabolic plan. Adaptive variations in the other enzymes may occur to alter alkaptonuria under certain conditions.

Katsch was also involved in a premature and erroneous proof of Garrod's thesis,

the supposed absence of an enzyme which oxidized homogentisic acid. The absence from alkaptonuric serum of such an enzyme, and its presence in normal individuals was reported by Gross (1914). Katsch and Stern (1926) said it was not an enzyme that was absent in alkaptonuria, but an inhibitor that was present, although the functional result was the same. Any difference between normal and alkaptonuric urine was promptly denied by Lanyar and Lieb (1929), who showed that poor pH control produced these results, but this first "proof" of a missing reaction in an hereditary disease was already widely publicized. The disproof has not yet caught up with the "proof" cited in many standard texts.

Experimental Alkaptonuria: Support for Garrod's thesis of a blocked normal metabolism gradually evolved from numerous instances of homogentisic acid excretion by normal animals under somewhat abnormal conditions (see Knox, 1955, for references). The premium associated with the identification of homogentisic acid in urine led Abderhalden to the heroic extremity of administering 50 g. of tyrosine to an assistant. A trace of homogentisic acid was excreted. When the assistant refused to repeat the test for confirmation, Abderhalden himself took 150 g. between 9 a.m. and noon. He did not excrete any homogentisic acid (Abderhalden, 1912). Excretion of homogentisic acid was observed in some rats who survived toxic doses of phenylalanine plus ascorbic acid and in rats on certain protein or amino acid deficient diets. The most physiological experiment was the eventual development of alkaptonuria in rats fed phenylalanine for at least three weeks (Papageorge & Lewis, 1938; Lin & Knox, 1957b). Contrary to the widespread impression, homogentisic acid excretion was found only once in scorbutic guinea pigs fed tyrosine (Sealock & Silberstein, 1940) and is not a regular finding in scurvy. Ascorbic acid is not directly concerned with homogentisate oxidation (Knox, 1955). The most ingenious example of experimental alkaptonuria was that produced in guinea pigs by dosage with α,α-dipyridyl, an inhibitor of the iron-containing homogentisic acid oxidase (Suda, Takeda, Sujishi & Tanaka, 1951).

Spontaneous alkaptonuria has not been observed in any animals but man. There is a report of one rabbit which excreted a urine which darkened on contact with air and gave some qualitative tests for homogentisic acid. But homogentisic acid was not identified, and the animal died without offspring (Lewis, 1926).

Separate Enzymic Steps: Modern experimental approaches provided firm evidence for the normal metabolism of phenylalanine through homogentisic acid. Isotopic labelling experiments revealed that a rearrangement of carbons occurred in the course of the oxidation of phenylalanine to acetoacetic acid (Schepartz & Gurin, 1949). This rearrangement was like that which would be expected if a side-chain migration had occurred to convert a 4-hydroxyphenyl to a 2,5-dihydroxyphenyl compound. Homogentisic acid was not accepted as the normal intermediate in this metabolic path, however, until the step-wise series of enzyme reactions, including that forming homogentisic acid and that oxidizing it to known metabolites, were demonstrated *in vitro* in extracts of normal liver (Knox & LeMay-Knox, 1951; Knox, 1955). The completeness of our present knowledge of this pathway is illustrated by Fig. 5, every step of which is catalyzed by a known isolated enzyme.

The reactions of Fig. 5 also give a surprising answer to the pivotal chemical

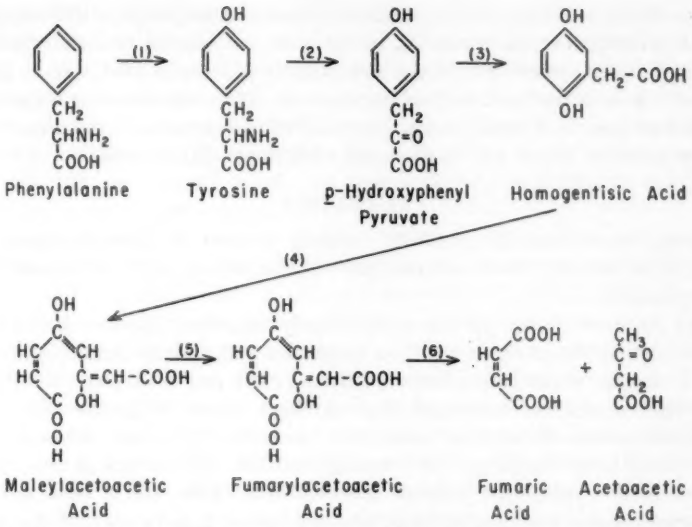


FIG. 5. The individual enzyme reactions of phenylalanine and tyrosine metabolism. The final products shown, fumaric and acetoacetic acids, enter the tricarboxylic acid cycle. Reaction (4) is the one inactive in alkaptonuria. The enzymes catalyzing each reaction are: (1) phenylalanine hydroxylase; (2) tyrosine transaminase; (3) *p*-hydroxyphenylpyruvate oxidase; (4) homogentisate oxidase; (5) maleylacetoacetate *cis-trans* isomerase; and (6) fumarylacetoacetate hydrolase (Knox, 1955).

question in the study of alkaptonuria. The intermediate quinol whose postulated existence was the basis of the metabolic studies does not exist unless as a transitory form on the enxyme surface.

Our present understanding leaves only a very small unexplained residue from all the observations made over two generations on this metabolic system. Three loose ends have persisted. These are the experiments, like those of Katsch, which suggested an altered or diminished activity of tyrosine oxidation in ketosis, and other poorly defined physiological states. It is known that ketosis leads to excretion of *p*-hydroxyphenylpyruvate, the precursor of homogentisic acid (Takeda *et al.*, 1952). The explanation may reside in the regulatory activities of metabolic adaptations. Second, it was observed that gentisic acid, formed from salicylic acid, was not further metabolized by the alkaptonuric patient as it was by normal individuals. Homogentisate oxidase, the enzyme missing in alkaptonuria, does not oxidize gentisic acid (Knox & Edwards, 1955), so the disturbed gentisate metabolism (based on non-specific chemical determinations of two very similar compounds) would indicate that there was another unsuspected abnormality of metabolism in alkaptonuria. The unified concept of the disease introduced by Garrod and now supported by all other evidence would then be incomplete. Third, the basic assumption about the nature of alkaptonuria, that the enzyme normally oxidizing homogentisic acid in liver and kidney is inactive in alkaptonuria, remained confirmed by direct investigation on the tissue of such

patients. As this is written, La Du, Zannoni, Laster and Seegmuller (1958) report that such an investigation was made. All of the other enzymes of tyrosine metabolism (see Fig. 5) were present in a biopsy sample of liver from an alkaptonuric subject, but that the homogentisate oxidase was inactive. There remain other complex and unexplained aspects of alkaptonuria, such as the pathogenesis of the pigmentation and the arthritis, which will be discussed with these clinical signs.

III. HEREDITY

Hogben, Worrall and Zieve (1932) correctly referred to Garrod's paper (1902) instead of his lectures (1908) or book (1909) as the primary study of the inheritance of alkaptonuria.

Single Recessive Factor: In the paper already described (Garrod, 1902), it was established that alkaptonuria was 1) a congenital and familial condition; 2) manifested in siblings whose parents were unaffected (with one exception); and 3) associated with an excess of consanguineous marriages among the parents. From these facts Garrod proposed that alkaptonuria was determined by a single recessive Mendelian factor, although he did not call it exactly that. By 1909 he knew of two pedigrees in which direct transmission occurred, those of Osler (1904) and of Orsi (1889). He intrepreted these as examples of the mating of a homozygous recessive with a heterozygote:

"When a recessive individual mates with an apparent dominant, who produces gametes of both kinds, a larger proportion of the offspring will be recessives, and we should expect that recessive children of a recessive parent, but whose other parent is apparently normal, will occasionally be met. Of such direct transmission of alkaptonuria from parent to child, the other parent not being alkaptonuric, two examples are known."

Fromherz included in his review (1908) the pedigree of the family he had observed with an "intermittently" alkaptonuric mother and three of twelve children alkaptonuric. Garrod had not included this pedigree as an example of direct transmission because of uncertainty about the diagnosis of the mother (Garrod, 1909). Fromherz collected fifty-eight cases at that time which confirmed the familial tendency of the disease, and incidently confirmed Garrod's statement that more could not be learned about the heredity of alkaptonuria until new cases were reported with adequate family studies. However, forty-five of the fifty-eight cases had been seen in the past fifteen years, so the prospect for more data was improving.

Toenniessen (1922) repeated a type of analysis used by Garrod (1909) to avoid the difficulties caused by lack of knowledge of the number of individuals in affected families and the failure to identify the proband for use in Weinberg's method. He used only the recently described, large families, with sufficient siblings "to give a good approximation of the statistical proportions of Mendel." His two large families plus the one of Fromherz (none of the parents were related) had a total of twenty-three normal and eight alkaptonuric siblings, nearly a 3:1 ratio. Adding to this those sibships numbering four or more from Fromherz' review, there was a total of thirty-five normal sibs and thirteen alkaptonurics, again nearly 3:1. This result confirmed Garrod's earlier evidence for the Mendelian segregation of the character. Toenniessen

also reprinted a pedigree from Umber and Burger (1913), one that Hogben *et al.*, later credited to Toenniessen, "which is the only pedigree besides that cited by Garrod showing direct inheritance." The pedigree will be described later. The 1:1 ratio of normal to alkaptonuric individuals in the second generation (four of each) with one affected parent would be expected with a dominant character as Hogben later interpreted it, but this ratio was actually cited by Toenniessen as proof of its recessivity, arising "through the cross RR x DR, and then in F_1 the results must be 1:1, so this pedigree gives the theoretical number."

The most complete and scholarly study of the inheritance of alkaptonuria is still that of Hogben *et al.* (1932). The number of recorded cases had grown to 120 by 1923 (Garrod, 1923) and then in 1932 to 151 cases included in the study by Hogben *et al.* Forty-two were isolated cases without reference to familial incidence or parental consanguinity. In forty-five fraternities with such information more than half had more than one affected sib. If the isolated cases were included, there was still more than one affected sib in nearly one-third of the fraternities. The familial nature of alkaptonuria was thus evident from an incidence of a very rare disease among sibs that was "vastly higher than could be accounted for by pure chance." The observed number of cases in thirty-seven fraternities of sizes one to fourteen where complete information was available was compared with the expected number on a recessive hypothesis (corrected for ascertainment only through affected individuals). There was no significant difference between the observed number of 66 and the expected number of 61.9, and the incidence therefore fit the hypothesis of a recessive gene. The large number of affected sibships with normal parents, and of normal sibships with one affected parent, confirmed the recessive character. This was further borne out by the fact that 42 per cent of the sixty-three patients whose parents' relationship was ascertained were the offspring of consanguineous matings. From this "highest incidence of consanguinity" that had been found, appropriately in the "rarest disease studied," they calculated the incidence of alkaptonuria to be between one in a million and one in ten million in the population. The validity of this evidence for a simple recessive inheritance in the majority of families with alkaptonuria does not come into question when another mode of transmission is considered for a small number of additional cases.

The unequal sex ratio of patients with alkaptonuria did not find an explanation in the hereditary mode of transmission. A higher proportion of males than females had been noted by both Garrod and Fromherz. Garrod had compared it to the similarly unequal sex ratio observed in cystinuria. Hogben *et al.*, found one hundred males and forty-six females among the recorded alkaptonurics of known sex. They discarded the hypotheses of sex-linked inheritance or lower penetrance in females because of the nearly correct Mendelian ratios observed, and they also found no clinical evidence that the condition might be semi-lethal in females. They showed how at least part of the disproportion could be ascribed to the fact that males were often the probands in affected sibships. Their conclusion was similar to that suggested by Niemann (1876) for the preponderance of males with cystinuria: that sociological factors accounted for the apparent disproportion. These included reticence about micturition in women and the more frequent subjection of males to medical and life insurance examinations.

This suggestion also implies that many cases are not diagnosed until the reducing action of the urine is found. Hogben *et al.*, noted that there were actually more females than males reported among infants, where the sociological factors favoring diagnosis of males were less important.

Direct Transmission: Five separate pedigrees showing direct transmission of alkaptonuria had not been included in the above study. These were separately discussed, and though exceptions could be taken to several of them, that of Pieter (1925), in particular, was considered to *compel* the recognition of a dominant form of alkaptonuria. The major position taken by Hogben *et al.*, in this discussion was that the direct transmission of such an extremely rare disease by the mating of a homozygous recessive individual with one who was heterozygous, as was suggested by Garrod and Fromherz, would be very, very unlikely to occur unless the mating was consanguineous. The observed pedigrees did not have evidence *for* the expected degree of consanguinity, and it was therefore assumed that at least some of them represented a dominant form of alkaptonuria. This controversial conclusion of Hogben *et al.*, is worth detailed discussion and reevaluatiom.

New information on the incidence of alkaptonuria, and new pedigrees of families showing direct transmission of alkaptonuria must be considered in this reevaluation. There is reason to believe that the incidence of alkaptonuria in the population may be one or two orders of magnitude less rare than Hogben *et al.*, estimated. Cases of alkaptonuria have continued to be recorded with increasing frequency until only those with unusual features are now regularly published. Twelve primary cases were diagnosed at a single clinic over a period of twenty-six years (Martin *et al*, 1955). Most importantly, the incidence of alkaptonuria in Northern Ireland, determined by complete ascertainment, has been estimated by Prof. A. C. Stevenson to be three to five per million individuals (unpublished). Not only is the possibility of matings between individuals homozygous and heterozygous for the alkaptonuria gene therefore much greater than Hogben *et al.*, assumed, but some subsequent studies have been made of the same pedigrees, and of some new ones, which showed direct transmission of alkaptonuria. These studies somewhat alter the evidence for the existence of a dominantly inherited form of the disease.

Families with Directly Transmitted Alkaptonuria: Personal communications from the several authors to Garrod were cited by Hogben *et al.*, as an additional source of information about the pedigrees of alkaptonuria. However, comparison of the data in the original reports with that used by Hogben *et al.*, showed that no significant new information was added. The pedigrees must therefore be evaluated on the data available in the literature. They are reproduced in Hogben *et al.* (1932) and some are brought up to date in Milch (1955).

Orsi (1889): This fragmentary report of "familial pirocatechinuria" contained no information except that the mother and her son and daughter were affected. There is little doubt about the correct diagnosis from the tests reported, despite the name given the condition. "Pirocatechinuria" is the Italian version of "brenzcatechinuria," which was used by Ebstein and Muller.

Osler (1904) (and others): These two brothers, originally described by Marshall and Futcher, were the ones on whom Osler made the first clinical diagnoses of ochro-

nosis. Their parents were unrelated, and the older brother (Futcher, 1898) had two sons (know to Osler), one of whom was alkaptonuric. No statement was ever made about the possible relationship of the older brother and his wife in the critical mating, that which produced the alkaptonuric son. However, another brother (there were actually seven sibs in the first affected generation) is now known to have married a first cousin (Milch, 1955), so the possibility of consanguinity in the critical mating is not precluded. A subsequent investigation of this pedigree revealed no addition cases of alkaptonuria besides the son in the second affected generation (Milch, 1955). The affected son in the second generation was one of five siblings. The others were normal. Depending on the son's identity, there were either three third generation offspring or six third generation and eight fourth generation offspring, all of whom were normal. The occurrence of one consanguineous marriage in the generation which directly transmitted alkaptonuria, its appearance in only one of five offspring in the second generation, and its failure to appear in the third and possibly the fourth generations, are new facts to be considered in determining the mode of inheritance operating in this pedigree.

Fromherz (1908): This family was omitted by Garrod and by Hogben *et al.*, because of the uncertainty of the diagnosis of the mother. In this large family of twelve children, two were alkaptonuric by actual test, and from the mother's report, one more was alkaptonuric, one not alkaptonuric and one uncertain. The parents were unrelated, and not knowingly related to other alkaptonurics, although the mother came from the same locality as some previously described alkaptonurics. The father was normal. The mother gave a history of having often noticed that her urine was dark or quickly became dark on standing. Fromherz regularly left flasks at the house for each member of the family and collected them several days later. Two were regularly positive—those from the two alkaptonuric children. On one day three were positive—the third was from the mother. On repeated testing, no further positive sample was obtained from her. Though Fromherz could find no evidence, motive or admission of a mixup, he could not take the finding as free from exception until it could be repeated with the mother isolated in the hospital, or with the younger alkaptonuric child, who slept with the mother, out of the house. He suggested, however, that the mother might represent a case of "periodic alkaptonuria." Herein lies whatever importance the pedigree has, since other instances of intermittent alkaptonuria have been descrived, though not recently, and the documentation of them did not convince Garrod that they existed (Garrod, 1902). Yet the possibility remains that an "incompletely recessive" form might exist, which in the heterozygous form would occasionally give rise to detectable amounts of homogentisic acid excretion. Against this is the fact that the single positive sample from the mother in Fromherz' pedigree contained the usual amount of homogentisic acid, "too much to have been introduced only by contamination."

Umber and Burger (1913): This pedigree, though attributed by Hogben *et al.*, to Toenniessen (1922), was actually observed by Umber and Burger (1913). The high incidence of arthritis observed in the affected members, and Toenniessen's use of it to support the hypothesis of recessive inheritance alkaptonuria have been described. One alkaptonuric in an otherwise normal fraternity of six married a normal,

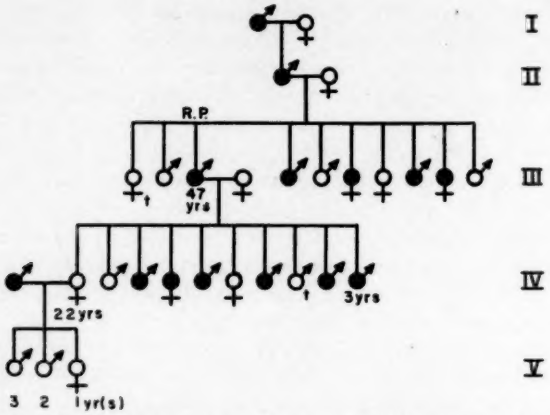


FIG. 6. Pedigree showing direct transmission of alkaptonuria, from Pieter (1925). The diagnosis of R.P., the propositus, is certain. A footnote said that alkaptonuria was *not* transmitted by any members of the third generation except R.P. (see text). ●, affected; ○, unaffected; †, stillbirth.

unrelated woman (both came from Holstein). There were eight offspring, four of them alkaptonurics. It should be mentioned that one of the four alkaptonurics in the second affected generation had two sons, both normal, and one of the latter had a son who was also normal. No new information about this family is known.

Pieter (1925): This was the remarkable pedigree which "compelled" Hogben *et al.*, to consider a dominant form of inheritance for alkaptonuria in certain instances. It is reproduced in Fig. 6. The original report, which described an agricultural family of mixed Spanish and Indian blood on the island of Santo Domingo, is of considerable interest in itself. The diagnosis of the proband is certainly correct. He was the forty-seven year old father (R. P., Fig. 6) of ten children, who was first diagnosed during hospitalization for removal of a bladder stone because his urine stained the bed sheets. Elaborate chemical tests, including the development of exposed photographic film in a solution of the urine (plus alkali, potassium bromide and sulfite) to give "a faint image after three hours," left no doubt that he excreted homogentisic acid. This use of photographic film anticipated the very useful test (1942) of Fishberg:

"Pour jeter un grain d'humour dans tous les ennuis que je lui ai causés, j'ai proposé à ce malade de s'improviser photographe. Il pourrait ainsi, lui dis-je, tenir boutique avec un minimum de frais généraux, car sa dépense en révélateurs pour plaques et papiers se trouverait réduite de beaucoup. En effet, comme on l'a vu, ses urines réduisent les sels d'argent. Cela tient sans doute à la nature chimique de l'acide homogentisique. . . . Son alcaptone serait donc un acide hydroquinone-acétique, et tout le mond connaît l'emploi de l'hydroquinone dans l'art de Daguerre."

Unfortunately Pieter was less solicitous and informative about other members of the family. He did not give the evidence of other diagnoses, whether by actual tests or by history or hearsay, nor any mention of relatedness of the spouses. He gave only those details which appear in the pedigree (Fig. 6), except for an illuminating footnote apparently missed by Hogben *et al.* In this he says of the proband's siblings,

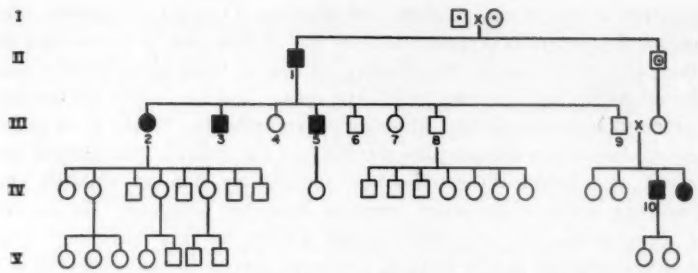


FIG. 7. Pedigree of alkaptonuria in Family 3 (Milch, 1955). Possibly this family was related to that of Pieter (Fig. 6). ■, affected male; ○, unaffected female; ⊡, status unknown.

four of whom were alkaptonurics: "Aucun autre sujet de la III^{e} lignée (tous mariés et avec enfants) n'a produit des alcaptonuriques." The presumed dominance of the disease in this pedigree was manifested only in the descendants of *one out of the five affected individuals*! An alternative explanation of the apparently direct transmission by one of five siblings would be a certain degree of consanguinity in the marriages of agricultural people on an island. Some evidence that inbreeding may have occurred can be found in a report of a possibly related pedigree (Family 3, Milch, 1955).

Milch (1955, Family 3): Fig. 7 shows the pedigree from Milch (1955) of a family which was "established with fair certainty" to be "descendents of the family earlier reported by Pieter. . . . Preliminary data tend to suggest that the 'original' male parent (II-1, Fig. 7) is in fact probably one of the males in Pieter's fourth generation." This particular relationship is difficult to believe, in view of the chronology. Since there is no reason to think that Pieter's report was long delayed between his observations and the publication, R. P. was nearly forty-seven years old in 1925, and the ages of his oldest and youngest children ("Pieter's fourth generation") were given as twenty-two years and three years. It is difficult to believe that even the oldest of these children would be a great grandfather, with seven greatgrandchildren, twenty-six years later when the pedigree was first described (Milch & Milch, 1951).

Whether or not the pedigree of Fig. 7 is connected with that of Pieter's (Fig. 6), it does illustrate the remarkable tendency, seen in some of the other instances of direct transmission, for the condition not to reappear in the third and fourth generations. It also proves that alkaptonuria genes were present in Santo Domingo in some concentration. Another, apparently unrelated, patient from Santo Domingo has also been described (Galdston *et al.*, 1952, Case III). If the pedigree of Milch is connected with that of Pieter's, it shows that consanguinity was not unknown in the family, since an unaffected member of the second affected generation (III-9) "married his (unaffected) first cousin." Two of their four offspring were alkaptonurics, strongly suggesting that at least here the usual recessive mode of inheritance was operating in this family said to compel belief in a dominant form. Dr. Milch now has reason to believe that his "original male" (II-1) may also have been married to a second or third cousin (personal communication).

Milch (1955) (Family 2): This family was described in part in several clinical studies before Milch (1955) restudied the family to correct discrepancies. Alkapto-

nuria occurred in four of nine children, the offspring of two normal parents who were first cousins. Alkaptonuria occurred in three out of four sibs in the second affected generation, but solely among the offspring of one of these alkaptonurics from the first affected generation who married his first cousin, and not among the five offspring of two other alkaptonuric siblings who married non-relatives. In the third generation, the three children of one alkaptonuric married to a non-relative were normal. Another alkaptonuric, also apparently married to a non-relative, had two children. One was normal and the status of the other "remains somewhat nebulous." He was thought to have alkaptonuria, but was never tested. Except for this "nebulous" case, the pattern of transmission in this pedigree conforms exactly to that expected of a rare recessive trait.

Other Pedigrees Showing Direct Transmission: Fifteen alkaptonuric individuals were found among a kindred of three hundred traced for seven generations (Hall, Hawkins and Child, 1950). The pattern of inheritance was evidently that of a simple recessive character, and this was supported by the occurrence of extensive inbreeding in the first five generations, and the presence in the area of about three hundred additional members of the kindred who were not studied. Klein (1953) stated that: "On rearranging the pedigree, the dominant transmission with three affected sibships of non-consanguineous parents, one of whom was likewise affected, becomes more evident." This is misleading. There were two, not three, instances of direct transmission, and in one of these the affected parent was known to have married his first cousin. The relationship of the wife to the affected parent in the second instance of direct transmission was unknown, since he lived in the early days of the settlement of the county. There were a total of only three affected sibships whose parents were not known with certainty to have been related. This uncertainty cannot be cited as evidence that they were not related, however, especially in view of the extensive inbreeding elsewhere in the kindred, the presence of an equal number of unstudied relatives in the immediate area, and testimony that at least one of these couples were related. Again it is observed in this pedigree that direct transmission stopped with the second generation. Wherever the pedigree is complete it illustrates recessive inheritance.

Cases 3 and 5 of Martin, Underdahl, Mathieson and Pugh (1955) each gave a history that their mothers had alkaptonuria. The sister of one of them had "funny" urine. No further details of the families were given, and as they stand the pedigrees are less informative than that of Orsi.

A pedigree at present unpublished (Khachadurian & Abu-Feisal) shows a total of seven alkaptonurics in four successive generations of a village family in Lebanon, an instance of direct transmission comparable only to that recorded by Pieter. However, investigation proved that not only were all affected individuals in each generation the offspring of consanguineous marriages, but that the population of the whole village was in some way related and many of them were familiar enough with the phenomenon of alkaptonuria to state positively who had it and who did not.

Dominance by Default: Ten pedigrees showing direct transmission of alkaptonuria are now known. So little is known about that of Orsi and the two described by Martin *et al.*, that they can only be enumerated. That of Fromherz is still unproved. That of

Milch (Family 2) and the large kindred described by Hall *et al.*, show typically recessive inheritance, with the instances of direct transmission accounted for by RR x RD crosses between first cousins, as was originally suggested by Garrod for the Osler pedigree. Of the remaining four pedigrees, on which a decision must be based, one is new (Milch, No. 3), there is new information about Osler's and Pieter's and only that of Umber remains as it was when considered by Hogben *et al.* Even in the latter pedigree the absence of alkaptonuria in the two sons and the grandson of one of the alkaptonurics, which Hogben *et al.*, suggested might be a chance occurrence, assumes a new importance in view of the almost uniform failure of directly transmitted alkaptonuria to appear later than in the second generation. It was not transmitted to three (or six) offspring of the third generation in the Osler pedigree, to nine in the third generation of the Milch No. 3 family, or to four fraternities each with an affected parent in the Pieter pedigree. The two instances with three or more generations affected depend upon the presence of the disease in the father and grandfather of Pieter's forty-seven year old proband, and in the "nebulous" but possible alkaptonuric in the third affected generation of Milch's family 2. In neither of these instances is there even evidence that the diagnosis was correct, and the latter family shows an otherwise typically recessive inheritance. In a recessively transmitted condition the failure to reappear in a third generation could be expected.

Inbreeding occurred at least in the family, when it was not known to have occurred in the critical mating, in the Osler and Milch # 3 pedigrees (and possibly by relationship with the latter, in the Pieter pedigree).

To the high probability of some inbreeding in the families showing direct transmission of alkaptonuria and failure of the disease to appear with certainty after the second generation, must be added the newer estimates of the incidence of the disease. On the basis of an incidence of five alkaptonurics per million in the population, about one in every 200 people would be heterozygous for the gene. A small but definite number of cases showing direct transmission of this recessive anomaly could therefore be expected to occur in each generation solely on the basis of chance. All of the ten pedigrees showing direct transmission of alkaptonuria are therefore most probably instances of recessive inheritance, none compel belief in a hypothesis of dominant inheritance, and the choice of a dominant over a recessive inheritance must be based on information which is lacking in each pedigree instead of on positive facts.

The overwhelming evidence that the great majority of cases inherit alkaptonuria through a single autosomal recessive gene is also supported by a wealth of biochemical evidence for a single inactive enzyme reaction as the basic cause of all the disease manifestations. This evidence should also be considered in favor of a simple hereditary transmission. The same kind of a biochemical lesion could also be caused by an allele with more serious effects, like the "incompletely recessive" variant of the cystinuria gene. But is is unlikely that a more complex action involving several genes would have such a precise biochemical effect. It should also be noted that the cases showing direct transmission of alkaptonuria do not differ biochemically in any known way from the great majority of cases. The success of Garrod's concepts about alkaptonuria is primarily due to his insistence on maintaining the simplest possible hypotheses for both the heredity and the metabolic abnormality. An hypothesis recently pro-

posed of an "incompletely penetrant dominant gene which co-exists with at least one other pair of modifying gene factors" (Milch, 1955), could, of course, be fitted to cover all cases. Such an hypothesis is undesirable, however, and for the great majority of cases, unnecessary.

IV. PATHOGENESIS OF CLINICAL SIGNS

The diagnosis of alkaptonuria is usually said to rest upon a classical triad of darkening urine, pigmentation of cartilages and arthritis. In actual fact, the diagnosis more often rests upon the correct interpretation of an atypical test for sugar in the urine, and only in later life are the pigmentation and arthritis at all prominent. The reducing action of the urine, caused by something which turned dark in alkali, was the only clinical sign of the disease known until the turn of the century, and it is even now sometimes repeated that the condition is completely benign.

Pigmentation: Not until a decade after the work of Wolkow and Baumann in 1891, by which time over forty patients had been reported, was any mention made of the second most characteristic sign of alkaptonuria—the pigmentation of the tissues. The black tissues had been observed, however, though not during life and not knowingly in association with alkaptonuria. Virchow himself reported the first case as a pathological curiosity. Autopsy of a sixty-seven year old man who died with generalized anasarca had disclosed, "at the first cutting of the thorax," that the cartilages were coal black, a condition to which Virchow gave the descriptive term "ochronosis" (Virchow, 1866). Almost nothing is known of the patient's clinical history, but this patient, like that of Bödeker, also had "arthritis deformans," especially of the knees. Virchow likened the black deposits and osteophytes in the joints to the tophi of gout. Cartilages and tendon insertions in the bones all over the body were stained black to light grey. Under the microscope the tissue clearly showed intercellular distribution of brown or yellow (ochre) pigment, from which the name was derived. Since the origin of the pigment or the factors directing it to its specific location in the mesenchymal tissues remain unknown, it is interesting to note that even the arterial intima, and especially the sclerotic aortic plaques in Virchow's case, were strongly pigmented. The colored parts of the cardiovascular system, like the colored cartilages, had degenerated to some extent.

The second case of ochronosis was reported twenty-five years after that of Virchow (it was presented in a "Festschrift" in 1891 honoring Virchow's seventieth birthday). Others soon followed, and the total of six were carefully reviewed by Albrecht (1902), when he added the seventh. These were pathological studies and very few clinical details were given, but in two of the cases a history of long-standing melanuria was reported. There had also been at least two autopsies on known alkaptonurics by that time (Osler, 1904), but they had included no mention of ochronosis (the ages of these patients were twenty-nine and forty-three years). It remained for Albrecht (1902) to demonstrate the connection between alkaptonuria and ochronosis. His patient had grey-blue ears "like dilated veins," and his urine turned dark on standing. He died with miliary tuberculosis soon after hospital admission, and when section revealed ochronotic cartilages, an heroic attempt was made to isolate homogentisic acid from the 20 ml. of clear, yellow urine found in the bladder (Zdarek, 1902). This

effort failed, but enough was learned of the properties of the reducing material present to recognize the case as one of alkaptonuria. This association of a clinical anomaly with a pathological curiosity was subjected to an ill-founded attack (Langstein, 1904) by a protege of Hansemann (1892), who had seen the two reported cases of ochronosis with melanuria. But confirmation of Albrecht's thesis by Sir William Osler (1904) silenced all further opposition, although Pick's invention of "exogenous ochronosis" caused by prolonged exposure to phenol had overtones suggesting it was also a subtle attack on Albrecht's thesis (Pick, 1906).

Osler, whose interest in this disease had undoubtedly been stimulated by Garrod's correspondence with him (Garrod, 1902), confirmed Albrecht's suggestion by reporting that two brothers who had been described earlier as alkaptonurics (Marshall, 1887; Futcher, 1898), could be recognized during life to have ochronosis of their sclerae, ears and across their noses: "There is no question that these are cases of ochronosis in long standing alkaptonuria and they support Albrecht's suggestion that the pigmentation of the cartilaginous tissues is associated with the remarkable disturbance of metabolism which we have heretofore only recognized by the changes in the urine. The condition is thus brought within the range of the clinical physician. *Fortunately it is not of much moment, so far as we know, and in the recorded cases there have been no symptoms directly due to the alkaptonuria*" (Osler, 1904). At least one of Osler's patients, from his own description, had disabling arthritis.

The pigmentation, as such, is not a really prominent sign during life, and is a minor cosmetic affliction. It accumulates slowly and has rarely been mentioned in patients less than forty years of age, though it can be seen earlier in the eye (Smith, 1942). In time, the pigment collection can become very dark. That located in the sclerae at the rectus insertions was once mistaken for a melanosarcoma and the eye enucleated (Skinsnes, 1948). When alkaptonurics have had urinary stones for other reasons, some of these stones were black (e.g. Martin, 1955, Cases 3 and 6). Like other melanin pigments of the body this one is a high polymer that can be dissolved in alkali and precipitated with acid—along with the proteins to which it is bound. It has been alleged that it is different from the usual melanins, since the oxidation product of chemically pure homogentisate has an absorption spectrum (Milch *et al.*, 1957) different from that of hair or choroidal melanin (Stein, 1955). But this assumes an identity between the pigment formed by autoxidation of pure homogentisic acid and that accumulating in the body tissues which is entirely inferential. Such autoxidation may occur in the tissues, since a few cases with similar pigmentation allegedly due to chronic phenol exposure are known (Pick, 1906). The deepest interest in the pigmentation concerns its possible causal relation to the degeneration of the cartilages and of the other tissues where it is concentrated.

Arthritis: It was casually mentioned in the original reports that Bödeker's first patient with alkaptonuria had "neuralgia" of the lower lumbas spine for two years, unrelieved after three months' hospitalization; that Virchow's first case with ochronosis had "arthritis deformans" (the report contains a colored drawing of the knee joint); that one of Osler's patients had osteoarthritis and Heberden's nodes; and that several other of the earliest patients had chronic polyarthritis (e.g. Embden, 1893). But unless such an associated condition is both unusual and almost invariably pres-

ent, it is often not immediately related to the primary clinical syndrome. The arthritis of alkaptonuria is not unusual, except for a particular pattern of intervertebral disc calcifications seen in advanced cases. It resembles the common types and affects the spine and large joints, though differently in each individual. It is usually like osteoarthritis (degeneration of the articular surfaces), but it may be much more active and assume the nature of rheumatoid arthritis (acute inflammation with later ankylosis). The older part of the normal population commonly complains of a similar process in its milder forms, calling it lumbago, sciatica, rheumatics, stiffness, etc. The arthritis associated with alkaptonuria also develops late, like the pigmentation, patients were seen as children, long before any arthritic changes occurred. Thus the arthritis associated with alkaptonuria is neither an unusual complaint nor is it invariably present in the patients seen, and consequently it was not recognized as part of the disease till about 1907.

The possible association of ochronosis with other pathological changes was considered, however, even before ochronosis was recognized to be part of the picture of alkaptonuria. Heile (1900) suggested that ochronosis was part of a "gouty-rheumatic diathesis," because the reported cases had either joint or cardiovascular pathology, or both. Albrecht fully discusssed the problem: of the seven known cases of ochronosis, three had marked arthritic changes, and all had some disease of the heart or aorta. Yet he was forced to conclude that this was a chance association: "We see heart failure and senile or arthritic joint changes of different degrees occurring together so often without ochronotic pigmentation, that we cannot accept Heile's almost unsupported suggestion." But one of the next cases of alkaptonuria to be reported (Gross & Allard, 1907) had such far advanced arthritis, with ankylosis of both upper and lower limbs, that he was nearly helpless. In Umber's (1913) family containing five alkaptonurics, all the affected members had arthritis. Umber was a specialist in arthritis, and his insistence that it was part of the disease was heeded. Of forty-six patients for whom Hogben *et al.*, (1932) gave some clinical details, twenty-four had some form of arthritis. We now know that in time arthritis develops almost invariably in all alkaptonurics. The consequences are often severe and painful, and may lead to a completely bed-ridden existence in later life. Pomerantz *et al.* (1941) have given one of several radiological descriptions of the lesions. Osler's statement that the disease "is not of much moment" or Garrod's strong belief in its benignity (so that he listed this as one of the cardinal though non-essential attributes of the "inborn errors"), simply point up the difficulty for even practiced observers to recognize clinically each of the pleomorphic consequences of an hereditary disease.

In view of the difficulty of identifying all facets of an hereditary disease, the records of these patients should be continuously scrutinized for unrecognized, late developing complications and for complications of less than invariable incidence. A cursory survey of the published cases of alkaptonuria reveals, for example, that the cardiovascular system, whose elements are known to be pigmented, often is the site of pathological changes, just as Heile indicated. Whether this process is more common, or is accelerated, in alkaptonuria can only determined by careful analysis.

The unknown pathogenesis of alkaptonuria remains a challenge. The explanation of the intermediate mechanisms is needed to connect a discrete gene abnormality

to a precisely located enzyme inactivity, then in time to pigmentation of cartilage and to arthritis. This challenge was provocatively phrased by Thannhauser (1929): "The arthritis of alkaptonuria is particularly instructive for general pathology, because we have here an endogenously produced metabolite, whose chemical structure is completely known, giving rise to a deforming joint disease. It is to take but one step to suggest that in the more common arthritis deformans of unknown etiology there is another endogenous metabolite acting like homogentisic acid and producing the joint changes." The knowledge of how a single hereditary abnormality ultimately produces its predictable arthritis in the rare alkaptonuric may be usefully applied to a very much larger number of patients with more complex and less well understood disorders.

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The Supernumerary Chromosome of Man

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IN A RECENT ARTICLE (Kodani, 1957b) the author reported a finding of three chromosome numbers in a group of Japanese testes and suspected that the numerical variation was due to a supernumerary chromosome. In another group of Japanese testes analyzed since then, the same three numbers have been observed again, and critical comparisons of the three caryotypes have clearly demonstrated the occurrence of a supernumerary chromosome in some of the testes.

The numerical polymorphism has thus far been reported only in Japanese. However, since the condition may occur in other ethnic groups, the chromosomes in the sex cells of White testes have been studied and compared with Japanese. This report is a detailed description of White and Japanese testes with the primary purpose of demonstrating the supernumerary chromosome of man.

MATERIALS AND METHODS

Twenty-three testes, eight from Whites and fifteen from Japanese, have been analyzed in this study. The latter were taken from epididymitis patients in a manner previously described (Kodani, 1957b). The testes from Whites were obtained from prostate cancer patients by total orchidectomy. Immediately upon removal, the testes were cut into pieces about the size of match heads and immediately fixed. Fixation was accomplished in two steps, pretreatment and postfixation. The pretreatment reagent was a mixture of equal volumes of a 1 per cent solution of chromic acid and a 3 per cent solution of potassium bichromate. (This solution is designated as K-12). After a one and one-half hour treatment in this solution, the pieces were transferred and allowed to remain in a mixture of equal volumes of 4.5 per cent chromic acid and 1.5 per cent potassium bichromate (K-24) for 17 to 20 hours. The latter mixture was always prepared immediately prior to use to insure its fixing power. During fixation the solutions were maintained at room temperature. After fixation the specimens were thoroughly washed in running water and made into Feulgen squash preparations.

Pretreatment with K-12 solution for the time specified above produces similar effects in the metaphase cells as hypotonic saline solutions and water: In the metaphase cells pretreated with K-12 and fixed with K-24, the chromosomes become well dispersed by the squashing, whereas if pretreatment with K-12 is omitted, the spreading of the chromosomes is very poor. The author's method has been found to give in

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general better fixation and dispersion of the chromosomes than pretreatment with a hypotonic saline solution or water followed by treatment with conventional fixatives.

Some of the specimens were large enough to have part of them used for sections. After fixation in either Bouin or Navashin's, serial sections were prepared. For accurate chromosome counts, sections have been entirely inadequate.

ANALYTICAL PROCEDURE AND RESULTS

From each testis enough squash preparations were made, first, to find at least several good spermatogonial metaphases in which the chromosomes could be counted accurately, and secondly, to find at least fifteen first spermatocyte metaphases in which the structures of individual chromosomes could be observed clearly. In some testes as many as thirty metaphases and in one testis sixty metaphases were analyzed. In every testis the chromosome numbers counted in the primary spermatocytes and spermatogonial metaphases were consistent and agreed exactly with each other. There was, however, one exceptional primary spermatocyte in one of the Japanese testes. In this testis fifteen first meiotic metaphases were analyzed and one of them was found to have 49 chromosomes, while other metaphases all had 48 chromosomes. This exceptional metaphase will be discussed in detail in a later section.

In a preliminary way the chromosomes in every first meiotic metaphase in each testis were designated by alphabetical letters according to their sizes except the X- and Y-chromosomes. Since size alone does not sufficiently characterize the individual chromosomes, those of the same preliminary designation in different cells were then compared with regard to shape determined by the position of the centromere. If agreement was unsatisfactory, the preliminary designations were changed until pairs of chromosomes could be defined which from cell to cell corresponded in both size and shape. By this procedure the chromosomes, at first meiotic metaphase, were seriated for each testis. No apparent discrepancies were encountered in any of the testes.

Comparisons of the Japanese testes have shown (1) that the same 23 pairs of chromosomes including the X-Y pair occur in all and (2) that while in some testes no other chromosomes besides the regular 23 pairs are present, in others a small chromosome is present either singly or in duplicate in addition to the regular members. Thus the numerical variation previously reported (Kodani, 1957b) is confirmed in the present group of Japanese testes. Furthermore, the preliminary explanation for the numerical variation as due to a supernumerary chromosome has been substantiated. In the White testes two numbers, 46 and 48, have been found. Comparisons of caryotypes representing the two numbers with each other and with those of the Japanese testes have indicated that apparently the same 23 pairs occurring regularly in Japanese are also present in both caryotypes of Whites, and the extra pair found in Japanese of the 48-chromosome type is likewise present in White individuals of the same type.

The fact that Japanese included in the present study all had epididymitis and the Whites all had prostate cancer raises the question regarding the possibility that

the morbid conditions of the individuals might induce changes in the chromosome number in their testes. Dr. Masamichi Suzuki, formerly of the Atomic Bomb Casualty Commission in Hiroshima, Japan, to whom the author's sincere thanks are due, provided the author with testis specimens taken by biopsy from twenty Japanese with complaints of sterility. (These specimens are not included in the group reported here.) Some of the individuals were not exposed to the atomic bomb radiations and showed no sign of illness at any part of their bodies. Histological examinations indicated that their testes were normal. Among these apparently normal testes of the healthy individuals, the author found some with consistently 48 chromosomes and others with consistently 46 chromosomes. In view of the facts that the 46 and 48-chromosome types exist in the normal individuals and the chromosome number is consistent in each individual (46, 47, and 48) in the morbid sample observed here, it seems unlikely that the variation of the chromosome number found in the present study is due to the morbid condition of the individuals.

From a number of first meiotic metaphases observed in Japanese, two are selected from the 46-chromosome type, two from the 47 and three from the 48 for illustration. Figure 1 shows those of the 46-type in testis No. 534. Twenty-three elements are present, all unquestionably bivalents. The X-Y pair is identified by its characteristic asymmetry and the tapering distal end of one arm of the X to which the Y is attached. The sex chromosomes are conjoined in 60 per cent of first meiotic metaphases whereas they are separate from each other in the other 40 per cent. In either case the total number of chromosomes counted at metaphase I is always 46. This is substantiated by the same number found in spermatogonial metaphases in this testis, one of which is presented in figure 2.

Typical first meiotic metaphases observed in testis No. 636 are shown in figure 3. In both cells the elements, 24 in number, consist of 23 bivalents including the sex-determining pair and one univalent (designated sup. in the drawing). Since the autosomal bivalents are all structurally homozygous, the univalent chromosome cannot be a part of a chromosome broken off from an autosome. Nor can it be a fragment of the X or the Y, for these chromosomes are not any smaller than those of the 46-chromosome type. It is unquestionably an intact chromosome with its own centromere. In Fig. 3A this chromosome is located very close to a large bivalent, but this is believed to be accidental because in none of the other cells observed in this testis is the univalent chromosome paired with or located very close to any particular chromosome. The X-Y pair shows clearly the characteristic asymmetrical form in Fig. 3A. In Fig. 3B the pair is also found to be asymmetrical under the microscope, but this is not shown very clearly in the photograph, because the two ends of the pair are at different focal levels. Chromosome counts in spermatogonial metaphases (Fig. 4) all agree with first meiotic metaphases in showing the chromosome number in this testis to be 47.

From comparisons of the chromosomes of the 47-type (Fig. 3) and 46-type (Fig. 1), it can be seen that 23 bivalents in each match well. The univalent in the latter type is evidently an extra chromosome added to the regular complement of 23 pairs common to both types (see Fig. 12).

The extra chromosome is usually about the same in size as the Y-chromosome,

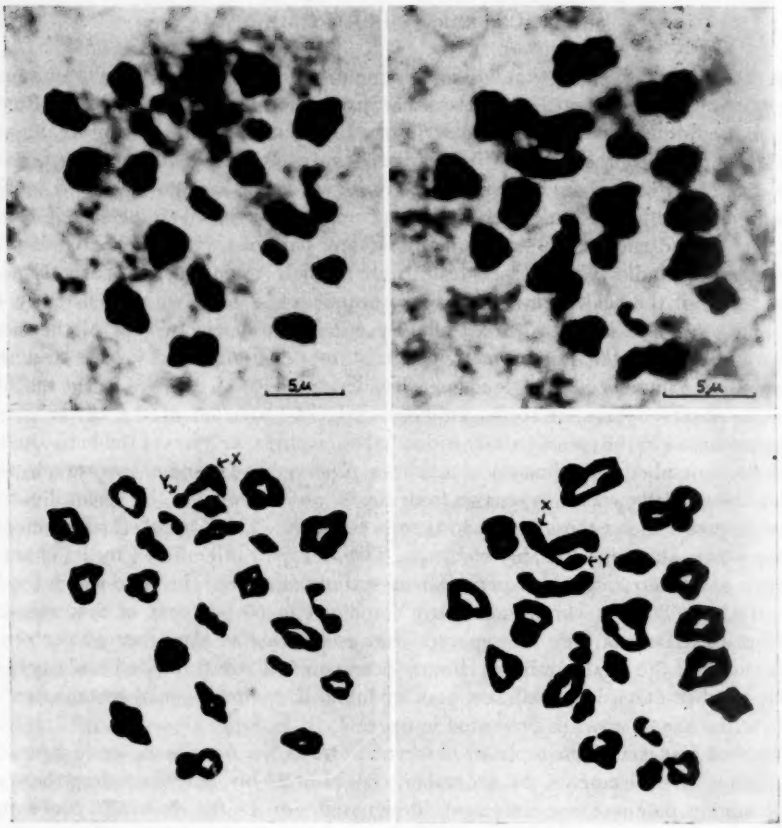

FIG. 1. Typical first spermatocyte metaphase of the Japanese testis no. 534. Note in both plates 23 bivalents including the asymmetrical X-Y pair. The chromosomes on the right figure are reproduced in Figure 12.

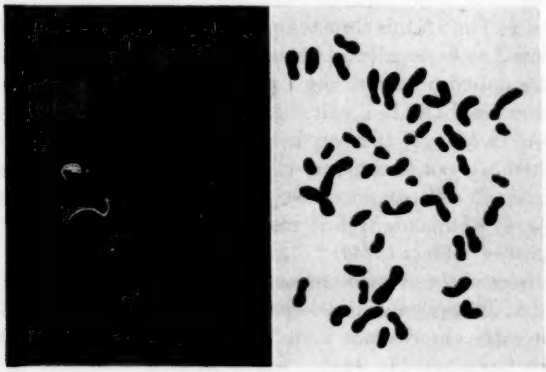

FIG. 2. A spermatogonial metaphase from the Japanese testis no. 534. The number of chromosomes is 46.

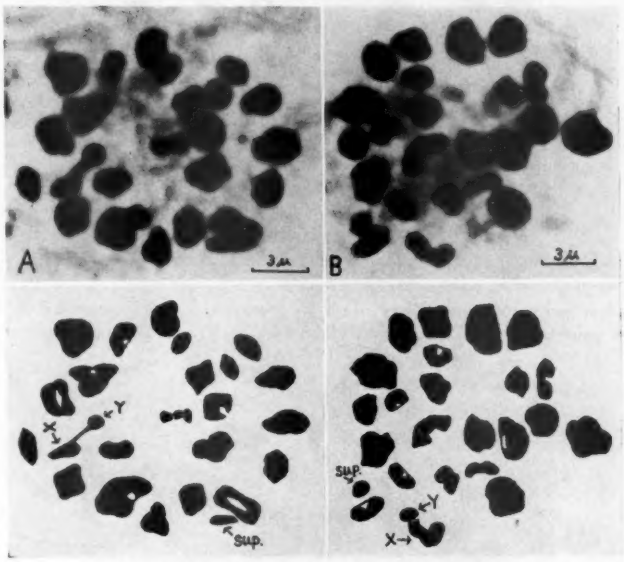

FIG. 3. Typical first meiotic metaphases of Japanese testis no. 636. Note in both plates the asymmetrical X-Y pair, 22 autosomal bivalents and a univalent supernumerary chromosome (sup.), making the total number of chromosomes 47. Plate A represents the 47-caryotype in figure 12.

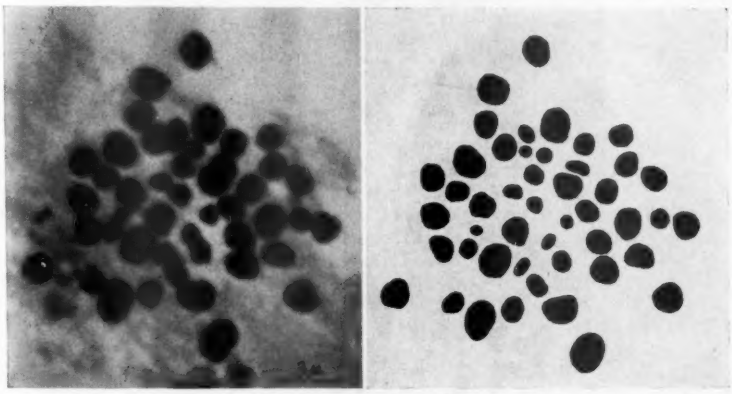

FIG. 4. A spermatogonial metaphase of the Japanese testis no. 636. Forty-seven chromosomes are shown.

although it sometimes appears slightly smaller. The exact shape has not been clearly shown in the 47-chromosome type of testes; in some cells it appeared more or less spherical while in others it was rod-shaped. However, its shape in terms of the relative lengths of its two arms has become known from the configuration observed in the testes of the 48-chromosome individuals which will be described below. The staina-

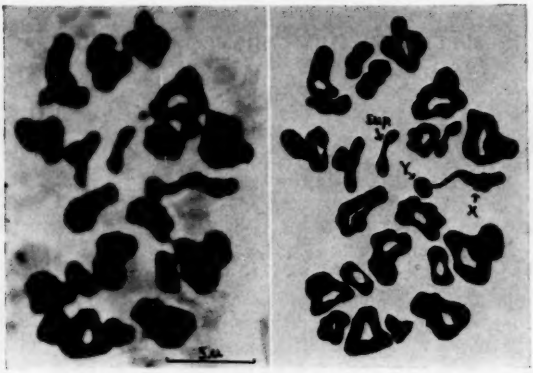

FIG. 5. A first spermatocyte metaphase of the Japanese testis no. 578, consisting of 22 autosomal bivalents and paired X-Y and supernumerary chromosomes (sup.). The total number of chromosomes is 48.

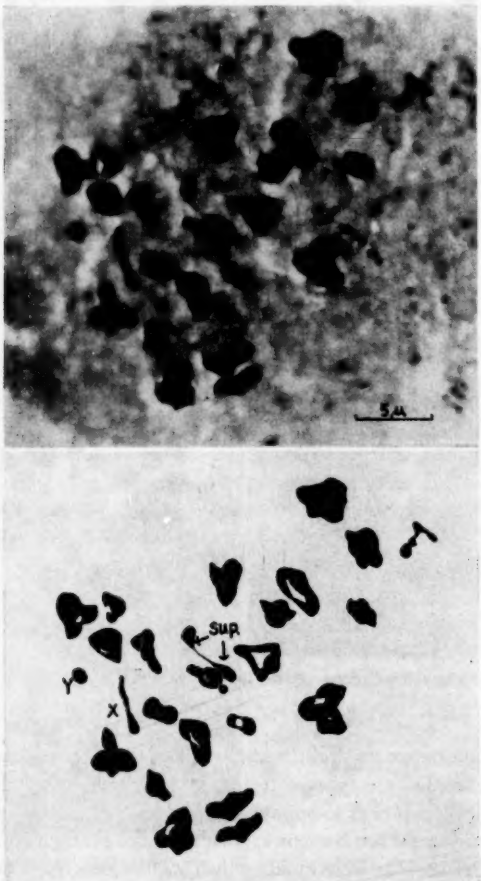

FIG. 6. Another first meiotic metaphase of the testis no. 578. Note that the sex chromosomes are not conjoined and the region of attachment of the supernumerary chromosomes (sup.) is extremely attenuated. Other autosomes are also distinctly bivalent. This figure is reproduced in figure 12 to represent the 48-caryotype in Japanese.

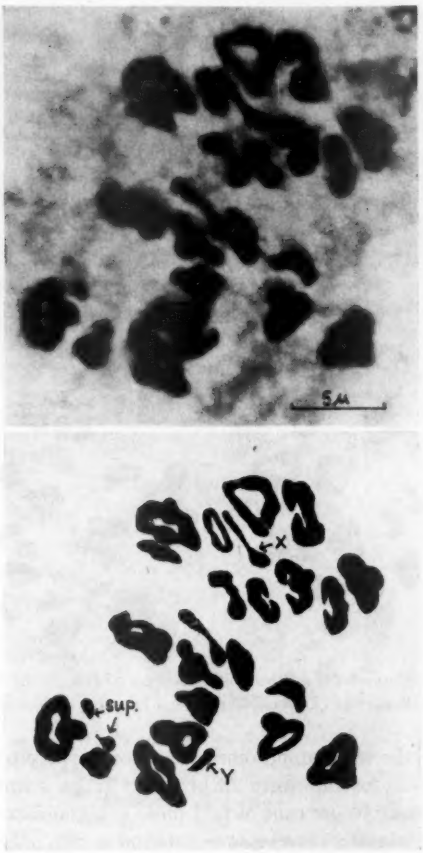

FIG. 7. This primary spermatocyte metaphase taken also from the testis no. 578 is different from those shown in the last two figures in that neither the sex-chromosomes are conjoined nor is the supernumerary pair (sup.). The total number of chromosomes is 48. For details see text.

bility and condensation of this chromosome are similar to those of the other autosomes.

Three first meiotic metaphases of the 48-chromosome individuals are presented in figures 5, 6, and 7. Twenty-four bivalents are present in figure 5. The X- and Y-chromosomes are conjoined forming an asymmetrical pair. One arm of the X to which the Y is attached is thinner and less chromatic than the other arm. This negatively heteropycnotic nature of its one arm, at first meiotic metaphase, is a unique but not consistent characteristic of the human X-chromosome (Kodani, 1957a). As in the 46- and 47-chromosome types, the X- and Y-chromosomes are thus paired in 60 per cent of first meiotic metaphases, while in 40 per cent of the cells they are separate from each other. Figure 6 illustrates one such cell with unpaired sex chromosomes.

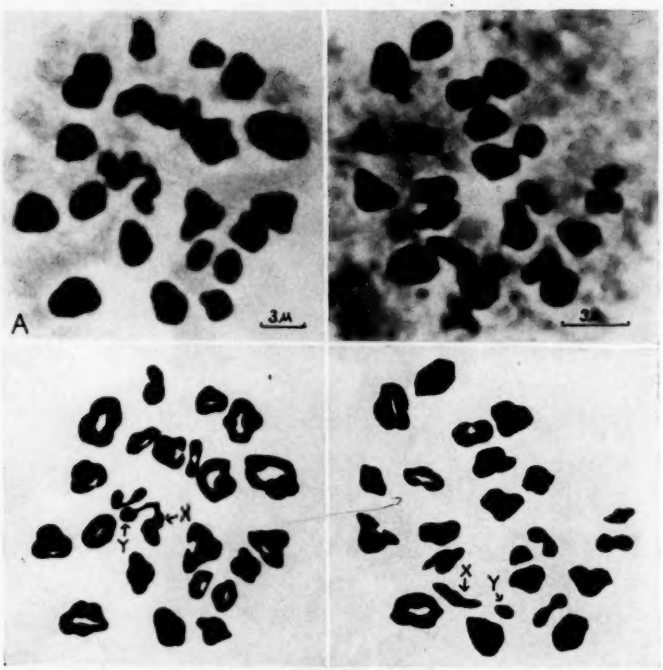

FIG. 8. First meiotic metaphases from the white testis no. 672. In A the X-Y and the autosomes are all paired, whereas in B the sex chromosomes are not conjoined while autosomal pairs are.

Here, also, except for the sex chromosomes, all autosomes are bivalent and the total number is 48. The 46 autosomes form 23 bivalents (Figs. 5 and 6), but this is not always the case: in about 10 per cent of first meiotic metaphases of individuals with 48 chromosomes a certain pair of autosomes is found as two univalents. One example is shown in figure 7. In this cell, besides the X and Y, two small autosomes of the same size (designated sup. in the drawing) are separate from each other instead of forming a bivalent. Observations of a large number of cells like this one have shown that the unpaired autosomes always belong to the same pair. Regardless of whether all chromosomes are paired or not, the total number is unquestionably 48 in the three kinds of cells in the two testes. Counts in the spermatogonia of these testes fully confirm the presence of 48 chromosomes. Comparisons of the first meiotic metaphases shown here with those of the 47-type such as the one shown in figure 3 indicate, as illustrated in figure 12, that the small pair of chromosomes labeled as sup. corresponds to the univalent chromosome in figure 3. Thus the small unpaired chromosome in the 47-chromosome-type is present in duplicate in the 48-type while entirely missing from the 46-type of testes.

Eight testes from Whites have been thoroughly analyzed to date. The first seven turned out to be of the 46-chromosome type. Two of the primary spermatocyte metaphases observed in one of these testes (No. 672) are shown in figure 8. Twenty-

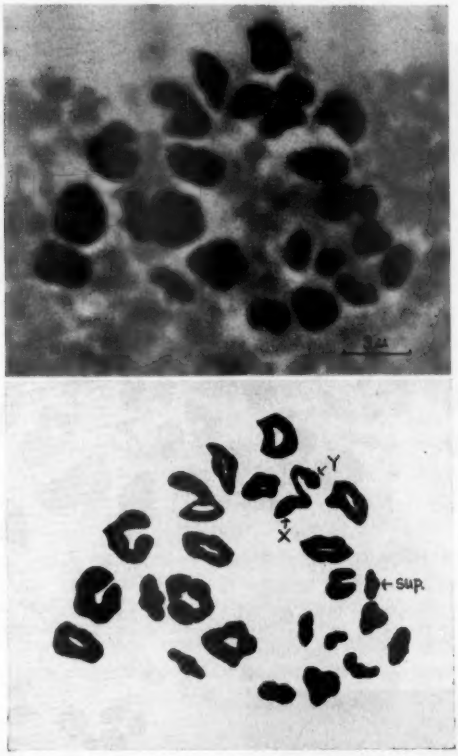

FIG. 9. A primary spermatocyte metaphase of the white testis no. 674, consisting of 22 autosoma bivalents and paired X-Y and supernumerary chromosomes (sup.). The total number of chromosomes is 48.

three bivalents are found in 8A, including the paired X-Y chromosomes. As in Japanese, the sex chromosomes are sometimes not conjoined at this stage. One example is shown in 8B. All spermatogonial counts have shown 46 chromosomes also. The chromosomes in the first meiotic metaphases shown here and in others observed in the same and six other White testes match satisfactorily those of the Japanese testes of the same type (see Fig. 12). No notable morphological differences seem to exist between the chromosomes of the two ethnic groups.

The last one of the group of eight White testes is of the 48-chromosome type. This specimen has been analyzed with special thoroughness and caution. Figures 9 and 10 represent first spermatocyte metaphases of this White testis. Twenty-four elements, all definitely bivalent, are found in these and other cells in this specimen. The matching of the chromosomes in these figures with those of the 46-chromosome type in Whites and the 48-chromosome type in Japanese indicates that an extra pair is present in the 48-type of Whites and that it corresponds to that in Japanese of the same type (see Fig. 12). As in Japanese the homologues of this pair in Whites are found some-

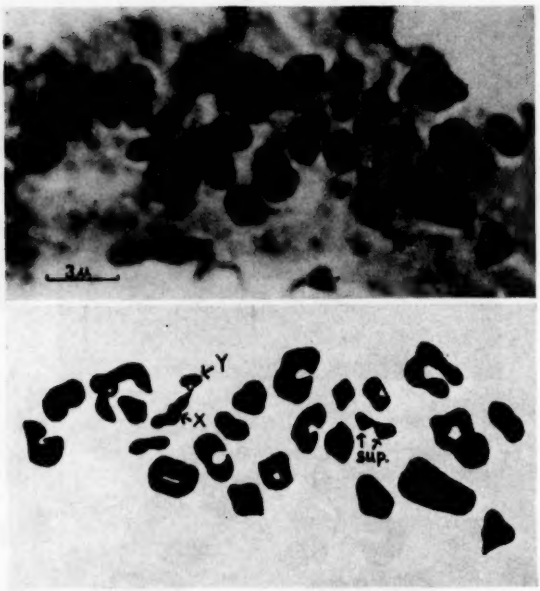

FIG. 10. Another first spermatocyte metaphase of the testis no. 674. The chromosomal constitution of this metaphase is the same as the one in the last figure. This metaphase is reproduced in figure 12 to represent the 48 caryotype in whites.

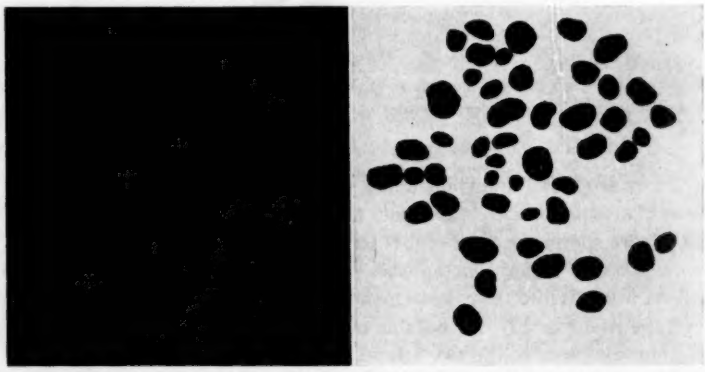

FIG. 11. A spermatogonial metaphase of the white testis no. 674. The chromosome number is 48. A large mass at the lower left corner is a sperm head.

times not conjoined at metaphase I, and when paired the attachment region is sometimes more or less extremely attenuated.

Several spermatogonial metaphases in this White testis, one of which is reproduced in figure 11, have clearly shown 48 chromosomes. The chromosomes in this particular metaphase and those shown in figure 4 represent the "balled-type" characterized by

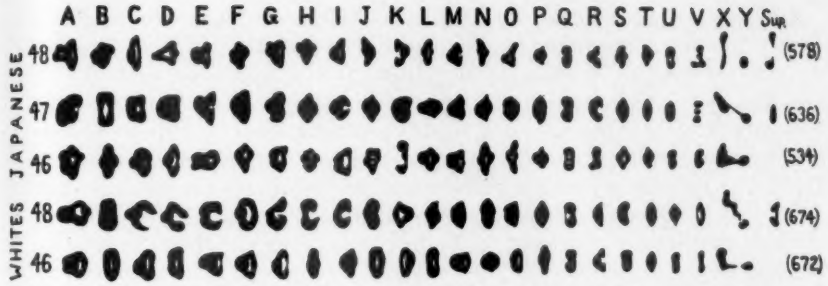

FIG. 12. First spermatocyte metaphases of three different caryotypes of man are compared to show the common set of 23 pairs (A-Y) found in all three caryotypes and the supernumerary chromosome occurring singly in the 47-type and in duplicate in the 48-type. The numbers on the left indicate the chromosome numbers and those on the right in parentheses the testis numbers. The metaphase representing testis no. 672 is not illustrated in this paper.

the overcondensation of all chromosomes into more or less regular spherical shape. This type of metaphase has been found to coexist with the "elongated-type" shown in figure 2, sometimes side by side in the same squash slide. When properly squashed the "balled" chromosomes become dispersed throughout the cell much more readily than the "elongated" chromosomes and are therefore far more favorable for accurate counting. Furthermore, the author's observations have shown that the chromosome numbers counted in the metaphase of the "balled-type" are constant within the testis. This fact indicates that a reliable conclusion could be drawn, if counts of a number of metaphases of this type all agree, as was the case with the author's materials.

DISCUSSION

Figure 12 summarizes the results of the analyses and comparisons of the three chromosome numbers found in Whites and Japanese. It represents the following three points: 1. The human species comprises individuals of three different chromosomal constitutions; 2. All individuals possess a common set of 23 pairs, but some individuals have either one or two additional chromosomes; 3. The chromosomes of Whites and Japanese are apparently alike in size and shape.

Widely varied chromosome numbers had been reported for men prior to 1923 (see Painter for references). Among these the one to be specially noted is 47 which was reported by de Winiwarter (1912) for the male. He believed that the human male has 46 autosomes and one X but no Y-chromosome. In 1923 Painter expressed the opinion that the numbers published before him were largely erroneous and he produced evidence to show that 48 was most likely the correct number. Two years later de Winiwarter and Oguma (1925) reported 47 again, but this number has never been generally accepted, because subsequent authors such as Evans and Swezy (1929), Minouchi and Ohta (1934), Shiwago and Andres (1932), Andres and Navashin (1936), Koller (1937), Hsu (1952), Mittwoch (1952), and Darlington and Haque (1955), have all published illustrations which, according to their beliefs, represented 48 chromosomes. The general impression thus created is that the original report of Painter was correct and that the chromosome number in man is consistently 48 in all male individuals.

Although it appeared that the question of the human chromosome number was settled, Tjio and Levan (1956) have recently demonstrated convincingly the presence of 46 rather than 48 chromosomes in cultured fetal tissues. Soon afterwards Ford and Hamerton (1956) reported the same number in testes from Whites in England. The latter authors expressed strong doubt concerning the occurrence in the human of any number other than 46. It is now clear, however, that the chromosome numbers 46, 47, and 48 occur in Japanese, and the first and the last, also, in Whites with little doubt that 47 will also be found in this group. It is interesting that the majority of the authors in the past had reported 48 which seems to be a much less frequent number than 46 among Whites. This was perhaps due to the fact that each author dealt with only a small number of individuals, although the possibility that some of the authors misinterpreted the number cannot be excluded.

Bender (1957) recently added another one of the 46-type, a White American female child, to the list of seven individuals of such type reported since 1956 by the authors mentioned above. The addition to this list of the seven White adults with 46 chromosomes described in this paper makes the total fifteen. In contrast to this number (15) the only 48-type individual found in Whites during the same period (1956–57) is the one reported here.

With regard to Japanese, among the 15 individuals in the present study, 9 have 46 chromosomes, 1 has 47 and 5 have 48. In the group of 21 individuals reported in the previous paper (Kodani, 1957b), the ratios of the frequency of the three numbers was 4:1:16. One individual who was then suspected to be of the 47-chromosome type was later proved to be so by the analysis of first meiotic metaphases. This is a different individual from the one reported in this paper, but the chromosomal constitution is essentially alike in both. At the time the 21 testes were picked for complete analyses from those which were preliminarily studied, the author exercised some selection in favor of those with the chromosome number larger than 46 in an attempt to find more individuals with 47 chromosomes. Therefore this group does not form a random sample. On the other hand, no such selection was made in the 15 Japanese and 8 Whites dealt with in the present study. Although these samples are too small to make a reliable estimate and comparison of the proportion of individuals with the three chromosome numbers, it seems that 48-chromosome individuals are more frequent among Japanese than among Whites. If this is actually the case, it would mean that the extra chromosome (sup.) found in the 47- and 48-caryotypes occurs more frequently in Japanese population than in White population. Studies of Whites and Japanese are being continued to find the answer to this problem and to others related to it, such as the adaptive value of this extra chromosome for the human species.

Apart from the multiple sex-chromosomes known in several species in the Mammalia, the numerical polymorphism of the autosomes in this group has been reported only in one species as far as the author is aware: Wahrman and Zahavi (1955) mention briefly, without cytologic demonstrations, a possible occurrence in the rodent *Gerbillus pyramidium* of two forms with different numbers of the autosomes inhabiting the same locality. The human is therefore the second one to be reported for such polymorphism.

In many species of the insects and flatworms (Melander, 1950), as well as in plants,

supernumerary chromosomes occur frequently. They do not seem to have obvious phenotypic effects. The 47th and 48th chromosome found in some men appear to be similar to the supernumerary chromosomes of other species. It is too early, however, to be able to decide whether the supernumerary chromosomes of man have phenotypic effects.

In some insects such as the Trimerotropi (White, 1951) the number of supernumerary chromosomes is constant in the same testis, whereas in other insects such as *Neopodismopsis abdominalis* (Rothfels, 1950) different numbers may be found in different cysts. Very little is known about the numerical consistency of the supernumerary chromosome in somatic cells. In the flatworm *Polycelis tenuis* (Melander, *ibid.*), it is absent from most of the somatic cells of the adult worm. In man, although the spermatogonial and spermatocyte anaphases are not yet fully investigated, it seems from observations of a large number of first meiotic and spermatogonial metaphases that the supernumerary chromosomes are transmitted with strict regularity from cell to cell in the spermatogonia: A total of nearly 300 first meiotic metaphases were analyzed in the nine Japanese and seven Whites of the 46-chromosome type, but in none of the metaphases did the chromosome number deviate from 46. Similarly in the two Japanese having 47 chromosomes, one reported here and the other reported in the author's previous paper (1957b), the chromosome number in forty first meiotic metaphases was consistently 47. In the five Japanese and one White of the 48-chromosome type, over a hundred primary spermatocyte metaphases were analyzed, and the number of chromosomes was exactly 48 in all except one metaphase. The exceptional metaphase was found in one of the Japanese testes. Among fifteen first meiotic metaphases studied in this testis, fourteen had 48 chromosomes and one had 49 chromosomes. This exceptional metaphase consisted of the conjoined X-Y pair, 23 autosomal bivalents and one univalent. There were no indications that fragmentation occurred in the X-Y or in an autosome. The univalent was unquestionably a whole chromosome, not a fragment, and its size appeared to be much larger than that of the supernumerary chromosome, but it was difficult to tell exactly to which chromosome of the complement it corresponded. This chromosome occurred probably by non-disjunction of one of the fairly large autosomes in a spermatogonial division. At any rate, the number of the supernumerary chromosome seems to be consistent in the human spermatogonia, i.e., one in individuals with 47 chromosomes and two in those with 48 chromosomes. In the somatic tissues nothing is known about the behavior of the supernumerary chromosome, since no one has yet reported observations of the chromosomes in cultures of tissues from individuals with 47 and 48 chromosomes in their gonads.

In the flatworms, and in many insects, the supernumerary chromosome is heteropycnotic, either positively or negatively, at one meiotic stage or another, and in some species it tends to associate itself with the X-chromosome at late meiotic prophase and first-metaphase (see White, 1954, for references). On the other hand, it may tend to associate itself with the autosome in some species, as in *N. abdominalis* referred to above where Rothfels (1950) observed one of the supernumeraries attached to one of the autosomes by what he called a chiasma. These phenomena have led a number of authors to speculate upon the original derivation of the supernumerary chromo-

somes from either the X-chromosome or an autosome (see White, 1954, and also Ray-Chaudhuri and Guha, 1955).

In man, the supernumerary chromosome has never been found to pair or tend to associate itself with any particular autosome. Evidently no genetic homology exists between this chromosome and the autosomes. This does not necessarily mean, however, that the supernumerary chromosome was not originally derived from an autosome. With regard to the question of the possible homology between this chromosome and the X-Y pair, it is certain that trivalent association of the three chromosomes does not occur at metaphase I, in either the 47- or 48-chromosome individuals. It is also certain that the supernumerary chromosome does not pair with the Y, for in individuals having 47 chromosomes a number of first meiotic metaphases have been observed in which the X and Y are not conjoined, but the supernumerary chromosome is not paired with the Y in any of these metaphases. The possibility still remains, however, that in these individuals the X may at times be paired with the supernumerary chromosome leaving the Y unpaired, because, due to the similarity in size between the supernumerary chromosome and the Y, it is difficult to ascertain which of the two chromosomes the X is paired with at metaphase I. Studies now in progress on the structure and behavior of these chromosomes during the meiotic prophase may shed some light on the problem. Although there seem to be no indications of genetic homology between the supernumerary and the sex-determining chromosomes at present, it is still possible that the former chromosome was originally derived from the latter. The origin of the human supernumerary chromosome remains obscure.

On the basis of observations of a number of first meiotic metaphase configurations in the testes of the 48-chromosome type, the centromere in the supernumerary chromosome in man has been located near its middle. As described in the last section, the two supernumerary chromosomes in the 48-chromosome individuals are found to be separate from each other in about 10 per cent of the first meiotic metaphases, but in the majority of the other 90 per cent of the metaphases they are conjoined terminally at one arm and frequently the attachment region is more or less strikingly attenuated. These are two unique characteristics of the human supernumerary chromosomes. Occasionally these chromosomes are conjoined at both arms and the attachments are always terminal. Since their behavior during the meiotic prophase has not been investigated yet, it remains to be seen whether the terminal attachments represent true chiasmata or something else (Cooper, 1944; Schrader, 1940). The similar terminal attachment of the X-Y pair at metaphase I presents the same problem. Cytologic studies of these problems are in progress now.

Andres and Navashin (1936) compared six mitotic metaphase chromosomes in two Russian and one Japanese testis and found that while three chromosomes (the fourth, sixth, and eighth) were the same in length in both groups, the three others (the largest three of the complement) in Japanese were considerably longer than the corresponding chromosomes in Russians. As pointed out by Stern (1949), even if their observations are correct the material is too limited to draw general conclusions concerning size differences of chromosomes between Japanese and Russians. In the study reported here, in which a far greater number of White and Japanese testes have been compared, no consistent differences in shape or size have been recognized. This does

not imply, however, that the chromosomes in the testes dealt with in this study are all completely homologous in structure, for some changes involving small segments and slight shifts of the centromere would not have been detected.

SUMMARY

The spermatogonial and first meiotic metaphases in 15 Japanese and 8 White testes were analyzed to determine the chromosome numbers and the sizes and shapes of individual chromosomes. Three numbers, 46, 47, and 48, were found among the Japanese, and two, 46 and 48, among the Whites. Comparisons of caryotypes representing the three numbers indicate that the numerical variation is due to a supernumerary chromosome. Some of the testes have one and others two supernumeraries besides a set of 23 pairs which is present in all of the testes. The chromosomes of Whites and Japanese are apparently alike in size and shape. Among the 15 Japanese, 9 have 46, 1 has 47 and 5 have 48 chromosomes, whereas among the 8 Whites, 7 have 46 and 1 has 48 chromosomes.

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Genetic and Physiological Aspects of a Family With Chronic Hereditary Lymphedema (Nonne-Milroy-Meige's Disease) and Hereditary Angioneurotic Edema

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INTRODUCTION

CHRONIC HEREDITARY LYMPHEDEMA is a rare condition which is usually localized in the lower extremities. The names of Nonne, Milroy, and Meige are frequently found as eponyms for this disease, although it was first described by Letessier in 1865 (quoted by Schroeder and Helweg-Larsen, 1950). In 1891 Nonne reported the condition in a German family, referring to the affectation as "elephantiasis congenita hereditaria." Milroy (1892) described this type of edema in a family in the United States, and found twenty-two cases among ninety-seven individuals in six generations. Meige (1898) referred to the condition as chronic hereditary trophoedema and reported a French family with eight cases (four females, four males). Thus, the condition has a variety of synonyms, but in the United States it is most frequently referred to as Milroy's disease. Milroy (1892) listed four characteristics of chronic hereditary lymphedema, as follows: congenital origin, limitation to one or both lower extremities, persistence of edema, and absence of constitutional symptoms. The edema was of a firm, persistent type, did not extend above the inguinal ligament, and was not accompanied by pain or tenderness. The condition is compatible with a long life; Milroy (1928) and Jennett (1956) partially traced separate families thirty-five and twenty-five years respectively after they were first reported. However, not all families show a congenital type of chronic hereditary lymphedema. The affected members of the family reported by Meige developed lymphedema at the time of puberty. Schroeder and Helweg-Larsen (1950) in a review of the literature divided the cases into two types: congenital, which is present at or shortly after birth, and tardive, which develops at a later age (from 9 to 28 years), usually around the time of puberty. Lymphedema also has been reported to affect the upper extremities along with the lower extremities (Burke, 1932; Drewes, 1939; Radner, 1946; Schroeder and Helweg-Larsen, 1950), but this is an unusual occurrence. In one patient the edema is reported to have extended beyond the inguinal ligament up to the mid-abdomen (Colp and Richmond, 1952). Some improvement in the edema has been manifested with aging (Meige, 1898; Schroeder and Helweg-Larsen, 1950) and Jopson (1898) and Panos (1956) each reported an instance in which the disease disappeared completely with aging. Hope and French (1908) described, in addition, asso-

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ciated lymphangitis-like "acute attacks" in some affected individuals. These attacks were characterized by chills, vomiting, high temperature, severe continuous pain and increased swelling in the affected extremity, and lasted for periods of three days to a week. Similar "acute attacks" have been described among affected members of other families, particularly those in whom the tardive type of chronic hereditary lymphedema occurred.

HEREDITARY ANGIONEUROTIC EDEMA

Hereditary angioneurotic edema is a rare condition and usually is inherited as a simple dominant. It is characterized by localized, indurated swellings surrounded by erythema varying in size and shape. It affects various parts of the body, including the face, particularly eyelids and lips, extremities, stomach, and glottis and larynx. It may be associated with gastrointestinal manifestations characterized by nausea, vomiting, abdominal colic and diarrhea. Death from asphyxia may result due to edema of the glottis and larynx. More males are affected than females (1.5 males: 1 female) and the onset may be at any age, but usually is around twenty years. Once the edema has developed, the tendency to recur remains throughout life. The etiology of angioneurotic edema remains obscure. Quincke (1882) believed that the basic mechanism causing this condition was a vasomotor disturbance of the blood vessels (angioneurosis) of the subcutaneous and submucosal tissues, eliciting increased capillary permeability and edema. However, the factors regulating and controlling the mechanism were not understood. Cockayne (1933) believed that there were two distinct types of angioneurotic edema. In the first and more severe type only the typical swellings appear in affected members of a family. In the second type, angioneurotic edema appears in some family members as one manifestation of allergic disease; in other members of the same family asthma, migraine, eczema, or urticaria may be manifest. He suggested that the basic cause of the edema was protein hypersensitivity, particularly in patients with associated asthma or urticaria. However, Fineman (1940) emphasized that sensitization to foods, inhalants, drugs, and bacteria does not seem to play an etiologic role in the hereditary type, but that there is some evidence that physical agents, such as trauma, and emotional stimuli act as exciting factors. Several families have been reported with a high concentration of angioneurotic edema (Osler, 1888; Ensor, 1904; Crowder and Crowder, 1917; Cameron, 1920; Dunlap and Lemon, 1929; Fineman, 1940). These cases are summarized by Gates (1946). A dominant autosomal type of inheritance is apparent in most cases, although penetrance does not always appear complete. Some family histories strikingly illustrate the severe manifestation of angioneurotic edema in affected individuals, as judged by the number who have died from glottic edema.

We have followed in detail a family group in which the tardive type of chronic hereditary lymphedema is manifested. In addition, a second family group which shows hereditary angioneurotic edema and is linked through one sibship to the first family group has been studied.

Family History

The complete pedigree is shown in Fig. 1. Normal spouses are omitted in most cases. Individuals are arranged from left to right in a sibship in order of birth. The

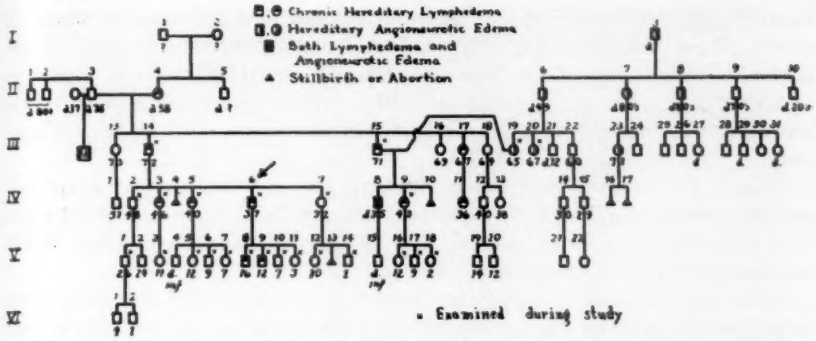

FIG. 1. Diagram of the families studied. See text for explanation of the pedigree.

numbers above each sibship line are to identify individuals within a generation. Numbers placed within symbols designate the total number of unaffected individuals in that particular sibship. Numbers beneath each symbol denote present age, or age at death when preceded by the letter "d". The patient, or proband, from whom this investigation stemmed, IV-6, is denoted by an arrow in Fig. 1.

Chronic hereditary lymphedema has been traced to the proband's paternal grandmother (II-4). No consanguineous marriages have occurred in this family. The parents of II-4, as well as her only sib (II-5), were killed during the Civil War, and no information is available for these individuals. For practical purposes II-4 may be considered as the progenitor of chronic hereditary lymphedema in this family, although we do not know if the disease in this individual was due to a mutation.

The proband's paternal grandfather (II-3), was married twice. He had twelve children with his first wife (six males, six females), none of whom had this trait, but only seven of these children lived past infancy. He had six children with his second wife (II-4), three affected with lymphedema and three not affected.

The age at onset varies from 11 to 42 years of age. The following ages have been specifically identified: III-14, 17 years; III-15, 20 years; IV-3, 42 years; IV-5, 13 years; IV-6, 14 years; IV-8, 17 years; IV-9, 26 years; IV-11, 11 years; V-8, 12 years V-9, 12 years. The age at onset in II-4 was reported by other relatives only as "early", and III-17 reported that lymphedema first began in her teens. Therefore, in the majority of individuals onset has been closely related to puberty, so that this family illustrates the typical tardive type of chronic hereditary lymphedema. Eleven years has been taken as the minimal onset age, or the age of first susceptibility for this disease in this particular family.

Twelve members of the family have been affected with chronic hereditary lymphedema. No cases of the disease are known outside of the direct lineage from II-4. Each affected individual (except II-4) is known to have an affected parent. There is no predilection for sex, six males and six females being affected. These data support autosomal dominant inheritance of the trait.

The segregation of children of affected individuals also is compatible with autosomal dominant inheritance. Eight affected individuals and their children who are eleven years or older may be considered. Among the nineteen children who have

passed the onset age of eleven years and who are descended from an affected parent, eleven (58 per cent) have been affected. This does not differ significantly from the number to be expected on the basis of autosomal dominant inheritance. Therefore, each child of an affected individual has a 50 per cent chance of developing chronic hereditary lymphedema. As far as can be determined at present penetrance is complete.

Personal data, including extent of affection, pre-onset factors, first symptoms, and therapeutic management of the edema were collected from affected members or their close relatives. Photographs of some affected individuals were taken at the beginning of the study and are shown in Fig. 2.

All but two (IV-3 and V-9) of the twelve affected individuals have both lower extremities affected, and the extent of affection is from the toes to the knees in most individuals. The member who most recently developed lymphedema was the proband's son (V-9). This boy developed swelling in the right lower extremity in 1956 and at present his left lower extremity is still unaffected. In most individuals the beginning of the swelling could be correlated with some event, such as an ankle sprain, a football injury, gun shot wound, or onset of menstruation. Only one extremity was affected at first, subsequently followed several months or more afterward by lymphedema in the other extremity for no apparent reason.

The greatest amount of swelling has tended to be in the legs, ankles and feet. Each individual has experienced a diurnal change in edema, with the least amount of swelling on rising in the morning and the greatest amount on retiring at night. Seasonal variations were reported by various members, although there was no constancy in such reports. Some stated the edema was aggravated by hot weather; others, by cold weather; and some felt there was no seasonal change. The affected women reported that they noticed an increase in edema premenstrually, followed by a postmenstrual decrease. The first symptom has been either swelling or pain, or both, in the affected extremity.

Further comment should be made on some individuals in the family. The proband's sister, IV-5, is one of the most severely affected members of the family (Fig. 2). Lymphedema increased in this individual following each of her pregnancies. However, it still does not approach an elephantiasis-like condition which has been reported in some affected individuals. At present, this subject notices transitory numbness in both of her arms, as well as a "bloated" feeling on arising. As yet no definite swelling has developed in her arms. Her youngest daughter, V-7, is now seven years old. This child has recently complained of her feet hurting her at times when she plays and runs, a symptom her mother also experienced in childhood. It is possible that this child may be manifesting the first signs of hereditary lymphedema, although no swelling has developed in her legs.

Another of the proband's sisters, IV-3, appears to be an atypical case of chronic hereditary lymphedema. She was asymptomatic until May, 1952, when she was 42 years old. At that time she twisted her right foot, and shortly thereafter sharp pain and swelling developed in the right leg, extending from the toes to the thigh. No edema has ever developed in the left extremity. This patient can partially control the swelling by following a restricted salt diet, whereas salt intake does not increase

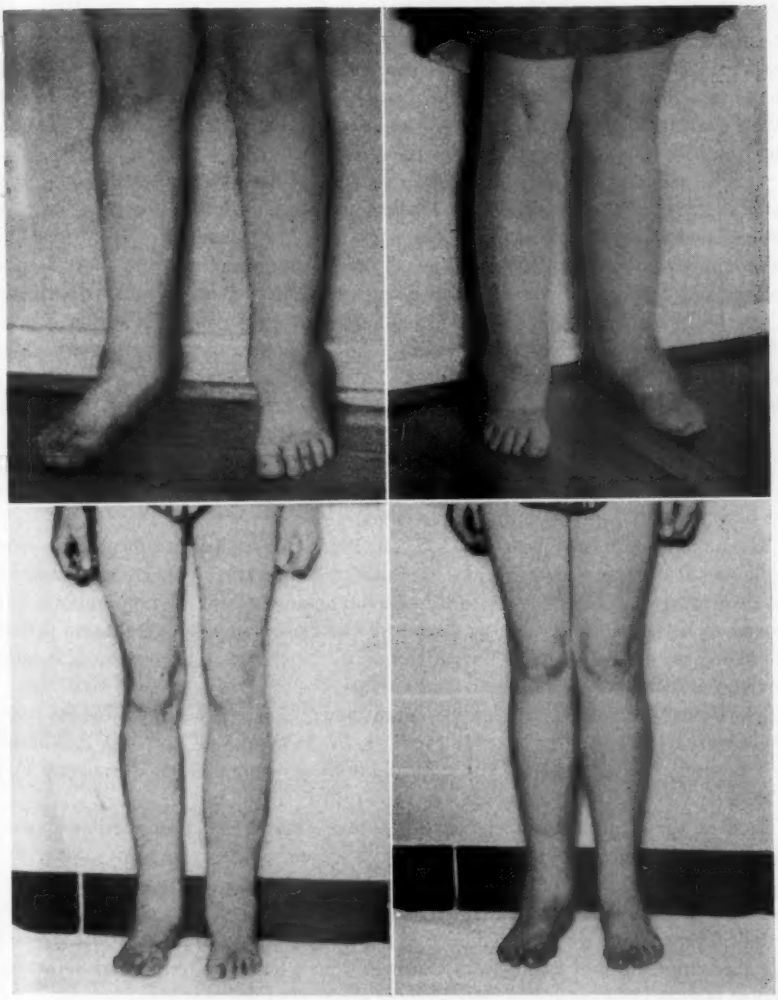

FIG. 2. Related individuals affected with chronic hereditary lymphedema. Upper left, the proband (IV-6); upper right, the proband's sister (IV-5); lower left, the proband's son (V-8); lower right, the proband's son (V-9). Pictures were taken prior to treatment with citrus bioflavonoid-vitamin C compound.

the lymphedema in her relatives. Other affected individuals resort to bandaging their legs or to wearing athletic stockings to help control the swelling. Some do not use any type of therapy.

The proband's cousin, IV-8, is unusual in that he inherited two rare genetic diseases. At seventeen years of age he developed chronic hereditary lymphedema of the lower

extremities. At twenty-four years of age he also developed angioneurotic edema which in time affected his face, pharynx, larynx, glottis, and stomach. His physician has reported that he died from suffocation due to glottic edema. He was thirty-five years old at this time. This individual inherited chronic hereditary lymphedema from his father and angioneurotic edema from his mother (Fig. 1). His parents report that one condition was not aggravated by the other.

Various members of the family have reported illnesses at different times which have aggravated the lymphedema. These correspond in description to acute "lymphangitis-like" attacks reported in the literature. Two members, III-15 and IV-8, have had several attacks diagnosed as erysipelas, but which were of short duration and were accompanied by increased swelling and tenderness in the affected extremity. After the birth of her first child, IV-5 suffered with phlegmasia alba dolens of her left leg, which caused increased swelling and discomfort in this extremity. Three members, III-17, IV-6, and V-8, have had attacks diagnosed by their physicians as phlebitis; at these times the affected extremity has become greatly enlarged, tender, and painful.

Until recently types of therapy reported in the literature have been of little benefit in managing chronic hereditary lymphedema, and bandaging the affected extremity has been the only form of control for swelling.

Recent reports (Greenblatt, 1955; Javert, 1955; Boines, 1955) indicate that citrus bioflavonoid-vitamin C preparations are beneficial in treating hemorrhagic conditions by decreasing capillary permeability. Bioflavonoid-vitamin C preparations have proven to be non-toxic agents, as shown by their use throughout gestation periods. Therefore, we decided to try this medication in large doses to determine if it might control the lymphedema in affected individuals.

Six affected individuals, four of whom are shown in Fig. 2, were selected for study. These were as follows (Fig. 1): III-14, IV-3, IV-5, IV-6, V-8, and V-9. As controls for this study, normal spouses or unaffected relatives were chosen. These were IV-2, IV-7, V-1, V-3, V-5, and the spouses of III-14, IV-5, IV-6, and IV-7.

Each of these individuals was asked to take circumference measurements each night before retiring of the following parts of the extremities: mid-foot, ankle, mid-leg, knee, mid-thigh, wrist, mid-forearm, and mid-upper-arm. Lymphedema was at its height at this time. Measurements were taken with a piece of non-stretchable umbilical tape which was referred back to the nearest eighth of an inch on a standard tape measure. Morning waking temperatures were taken to determine ovulation and days of menstruation were also recorded by premenopausal women.

A control period of six days, during which daily measurements and morning temperature readings were recorded, was followed by a thirty day therapeutic period in which individuals, in addition to collecting the aforementioned data, also took three capsules, in divided doses, of the citrus bioflavonoid-vitamin C preparation, commercially known as CVP capsules. Each capsule contained 200 mg. of vitamin C (twenty times the minimal daily requirement) and 200 mg. of citrus bioflavonoid compound. Following the therapeutic period, a final control period of six days was observed in which data were collected but no medication was taken.

Two control individuals (V-1 and the spouse of IV-5) were omitted from com-

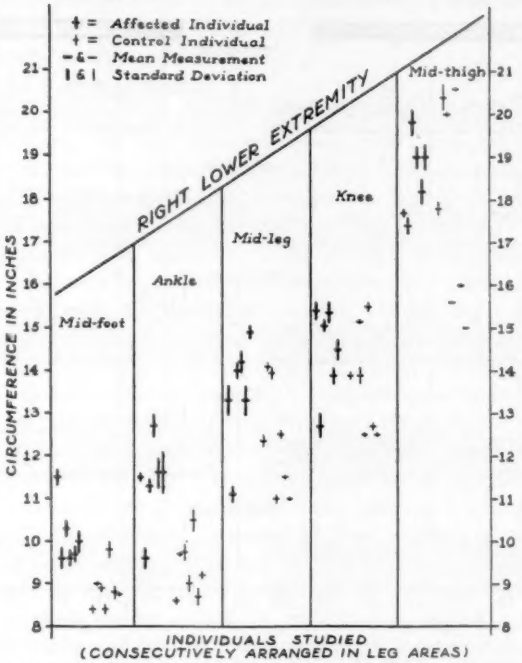

FIG. 3. Individual variation of the right lower extremity of affected and control subjects studied with citrus bioflavonoid-vitamin C therapy. No variation in measurements of the mid-thigh was recorded by two control subjects.

parison with affected individuals. In one case (V-1) no variation whatsoever was reported throughout the test period. Although this is possible, and desirable, it does not allow any human error during the six week measurement period; hence these data are not believed to be reliable. In the other case measurements were recorded too sporadically for a determination of a pattern. Therefore, seven control and six affected individuals were considered in the study.

The mean circumference measurements in inches for each point on the right lower extremity of both affected and control subjects are plotted in Fig. 3. Measurements of the left leg did not differ significantly from those of the right leg. The variations found during the period of measurements are expressed as the standard deviations. It will be noted from Fig. 3 that affected individuals tend to show a greater variation than control individuals.

The data for affected individuals were in definite contrast to the data for the controls. All affected subjects tended to show a great amount of variation during the test period, with a difference of as much as one and one-half inches or more being recorded in the size of the legs. Fig. 4 is an illustration of the measurements taken by IV-5. This great variation in affected subjects suggests that chronic hereditary lymphedema is a dynamic rather than a static condition and gives hope that bene-

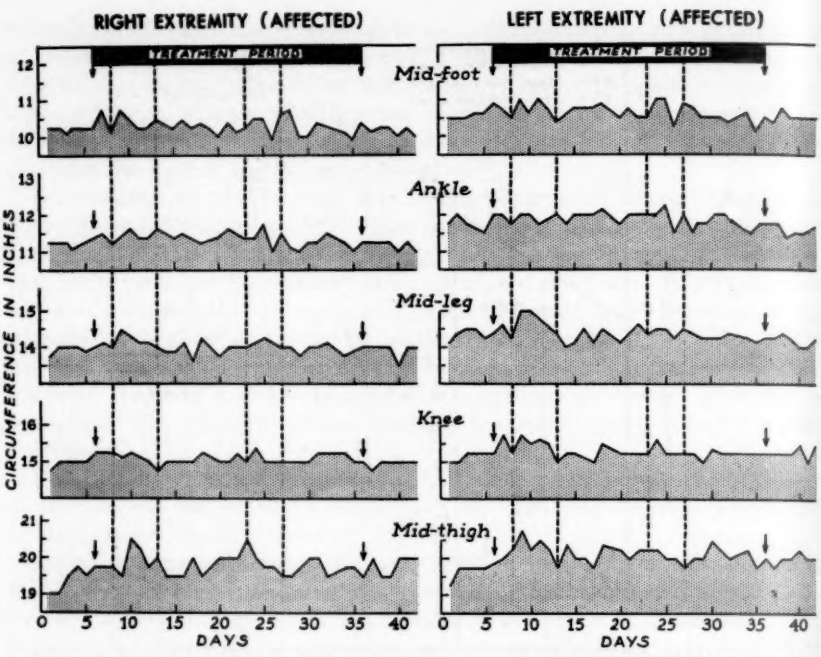

FIG. 4. Daily measurements of the lower extremities recorded by a subject (IV-5) affected with chronic hereditary lymphedema. The first and last six days of the experiment were control periods; during the thirty intermediate days 600 mg. each of citrus bioflavonoid and vitamin C were taken daily. Menstrual periods are outlined by dotted vertical lines.

ficial treatment may be found for it. It is notable that IV-5 observed variations in measurements taken during both the control periods and the therapeutic period of the test.

This individual had two menstrual periods during the time the CVP capsules were being taken. It is obvious from Fig. 4 that the greatest amount of edema is related to the menstrual periods. Measurements of all parts of the lower extremities show an increase in size of the individual part either one to two days premenstrually or during the first day or two of the mense, followed by a decrease in size of the extremities in the latter part of the cycle and postmenstrually. This was the only premenopausal affected individual studied.

Two control women also showed a slight premenstrual rise in measurements of the lower extremities. These observations suggest that the premenstrual increase in size of the extremities is due to retention of sodium and extracellular water by the tissues and is a physiological phenomenon, probably not related to chronic hereditary lymphedema.

We expected that there would be a significant decrease in the size of the legs during the therapeutic period if CVP was an effective control for chronic hereditary lymphedema. Three of six affected individuals did have a decrease in the size of specific

leg areas during the period of treatment, as follows: the mid-leg of III-14 and V-8 and the ankle of V-9. Therefore, further long-term studies are needed for full evaluation of CVP therapy in these individuals.

ANGIONEUROTIC EDEMA

Angioneurotic edema has been reported in four generations and is linked to the family with chronic hereditary lymphedema through IV-8, the paternal first cousin of the proband, IV-6. Investigation has revealed that several of his maternal relatives also suffered with the same condition.

Angioneurotic edema could be traced to the maternal great grandfather of IV-8. In addition, his other maternal relatives who were affected included his grandfather, a great uncle, great aunt, mother, aunt, and first cousin once removed (Fig. 1). Data on affected members were reported by III-19 and III-20.

Analysis of this family shows that angioneurotic edema has also followed a dominant autosomal pattern of inheritance with complete penetrance. Information is not as detailed for this trait as for chronic hereditary lymphedema, since only three of the eight affected individuals are still living. The age at onset was twenty-four years in IV-8, between twenty and twenty-five years in his mother, III-19, and thirty-five years in her sister, III-20. Other ages at onset have not been obtained.

IV-8 suffered with angioneurotic edema of his face, throat, and stomach. He was allergic to certain foods, and was described as "neurotic" by his physician. In addition, he also suffered with sinusitis. He expired at thirty-five years of age from strangulation due to a sudden attack of angioneurotic edema that involved the glottis.

His mother, III-19, reported that she suffered in childhood from attacks of severe colic that lasted from twenty-four to forty-eight hours. In her early twenties she began to have swelling of any part of her body, although her throat has only rarely been affected. Factors seeming to precipitate attacks include slight trauma, such as a bump against an object, or biting her lip, and certain foods, such as malted milk. She is allergic to dust and suffers from sinusitis.

Her sister, III-20, also stated that she suffers with angioneurotic edema of most parts of her body; however, her throat is only rarely involved. Usually her face, tongue, and lips are affected first. Attacks are precipitated by bumps or bruises, psychic factors, such as sudden fear or anger, and certain foods, such as scrambled eggs, fig preserves, and pineapple. Attacks seem to the patient to be associated with seasonal changes although they do not occur with any regularity. She also suffers with chronic sinusitis.

Angioneurotic edema was the cause of death of II-6 who expired at forty-nine years of age. The edema affected his throat as well as all other parts of his body. His father, I-3, was killed during the Civil War but had suffered with angioneurotic edema during his life.

Other relatives reported by III-19 and III-20 to have been affected are II-7 and II-8 who lived to advanced ages (in their eighties) before deaths from other causes. The daughter of II-7, III-23, is similarly affected. She is now 71 years old and has attacks of swelling, primarily localized to her hands and stomach. This individual

had two pregnancies, each terminating in stillbirths. In one case the child is reported to have been hydrocephalic.

One other individual, IV-9, may be genetically liable to both chronic hereditary lymphedema and angioneurotic edema. She has developed the former disease but as yet has not shown any sign of angioneurotic edema. Should she also develop this condition, her children (V-16, V-17, and V-18) who are now below the minimal age at onset could also be statistically liable to both of these rare hereditary traits.

DISCUSSION

Both chronic hereditary lymphedema and angioneurotic edema follow typical autosomal dominant inheritance with apparently complete penetrance in these family groups. Both have a delayed age at onset, so that many of the younger individuals who are normal at present may develop the condition at a later age. In these cases, children of an affected parent have a fifty per cent chance of being affected.

Surveys of the literature by Cockayne (1933) and Schroeder and Helweg-Larsen (1950) show that a greater number of women than men have been reported affected with chronic hereditary lymphedema. Cockayne (1933) suggested that a sex-linked gene might contribute to the more frequent occurrence in women, or inhibit the development of the disease in men. Schroeder and Helweg-Larsen (1950) have considered that the disease might be inherited in some families as a dominant autosomal trait, in others as a dominant sex-linked trait, and possibly as a dominant incompletely sex-linked trait in still others. In this way the excess of affected females over affected males is explainable. In the present pedigree sex-linked inheritance is ruled out by the male to male transfer of the gene in three instances (Fig. 1). Incomplete sex-linked dominant inheritance is certainly not very probable since crossing-over would have to be assumed in two individuals, but autosomal dominant inheritance is quite applicable.

Our records may help to explain the slight decrease in penetrance found for this trait in surveys of the literature. Previous reports indicate that the tardive type of chronic hereditary lymphedema develops in either the second or third decade. However, in one affected individual in our study, the condition was not developed until the age of 42 years.

In most individuals there has been some factor relatable to the onset of the disease. These factors vary widely in their nature, from trauma and inflammation, to a change in endocrine activity. The typical onset at puberty is indicative of correlation with increased metabolic activity in the individual. These factors may be of importance in the onset of edema in one leg, but it is difficult to see their importance in the subsequent development of edema in the other leg, unless metabolic or endocrine factors are of importance.

The basic cause of chronic hereditary lymphedema is undetermined. Schroeder and Helweg-Larsen (1950) have suggested that it is primarily due to a defect in the arterioles of the affected extremity, causing an increased amount of pressure in the capillaries, which leads to filtration edema; secondarily there is a change in capillary permeability. They base this belief on observations of arteriolar thickening noted in some biopsy studies done on affected individuals.

Recent reports by Dencker and Gottfries (1954) and Panos (1956) that glucocorticoids are of value in controlling the edema suggest that the primary defect is due to an increased capillary permeability, resulting either from abnormality of the endothelial cell of the capillary or of the intercellular cement.

The result of gene action may not necessarily be restricted only to the lower extremities. The effect may be on capillaries or arterioles throughout the body, but be more noticeable in the dependent areas of the body, particularly the lower extremities. The edema observed in our study seems to act hydrostatically, occurring first in the toes, feet, and ankles, and gradually extending upward toward the knee. A more generalized defect would explain those cases in which edema of the upper extremities as well as the lower extremities has been reported, and also the single case in which edema of the abdomen was noted. Jennett (1956) reports that three affected relatives, who reduced the amount of swelling in their legs by binding, then noticed some swelling of the face and hands. It would be interesting to check affected individuals for sacral edema at morning waking periods.

Our data for the upper extremities indicate greater daily variation in the affected individuals than in the unaffected individuals who were studied. This observation also suggests that the defect in chronic hereditary lymphedema is more systemic than localized, although actual edema may not be noted in all parts of the body.

We are not aware of previous use of citrus bioflavonoid-vitamin C compound to treat chronic hereditary lymphedema, but its trial is logical since other studies (Boines, 1955; Greenblatt, 1955; Javert, 1955) have indicated that it is effective in decreasing capillary permeability. However, it is apparent from our data that a thirty day therapeutic period, using large doses of CVP, is not sufficient to control the edema. The fact that there was some decrease in the edema in three of six affected individuals during this time indicates that more prolonged studies need to be done. Such studies are now in progress, and more definitive results, which will be reported in a subsequent paper, are being obtained.

We feel that it is important to evaluate fully the usefulness of CVP therapy because of its non-toxic nature and lack of side-effects for the affected individual who would need to use it for prolonged periods. However, it is also important to determine fully the value of glucocorticoid substances. Jennett (1956) treated two patients with glucocorticoid therapy for ten days, one with prednisolone (5 mg. orally four times a day) and the other with prednisone (10 mg. orally four times a day). There was no improvement in lymphedema in these individuals during this time. Either there was not sufficient time for a favorable response in these individuals or they are refractory to this type of therapy. Panos (1956) achieved favorable results in his patient by successively decreasing throughout a nine month period large doses of prednisone to a small maintenance dose (5 mg. orally per day).

It is of interest that the individuals who reported decreased measurements of the extremities while taking the CVP capsules included the oldest individual (age 72 years) and the two youngest individuals affected (ages 15 and 12 years). This suggests that changes which take place in the affected cases are not irreversible and that there may be hope for benefit in any affected individual no matter how long the disease has been manifest.

Data on morning waking temperature recorded by one affected woman (IV-5)

and one unaffected woman (IV-7) were indicative of the time of ovulation in these subjects during the therapeutic period. No increase in the size of the extremities was recorded by either subject at this probable time of ovulation. Basal body temperatures, however, have been shown not always to correlate with the time of ovulation as measured by other tests, such as the Spinnbarkeit (Speck and Halter, 1956). Also, data were not collected by these subjects for a sufficient period to show regularity in the rise and fall of basal body temperatures during several menstrual cycles. Therefore, no conclusions are justified in this regard.

In our study, as well as others reported in the literature (Dencker and Gottfries, 1954; Panos, 1956) increase in edema during the premenstruum has been reported for several women affected with chronic hereditary lymphedema. However, nearly all women have water and sodium retention just prior to the onset of menstruation (Anonymous, 1954) and as many as forty per cent of these may develop the complex of symptoms characteristic of premenstrual tension. Such periodic changes in fluid and electrolyte balance appear related to an increase in the ratio of estrogen to progesterone, which in turn may activate the production of antidiuretic hormone from the posterior pituitary, or mineralocorticoids of the adrenal cortex, or both, resulting in fluid retention.

Therefore, retention of excess fluid premenstrually by women affected with chronic hereditary lymphedema is most likely a physiological response not related to the defect causing chronic hereditary lymphedema. However, this response surely aggravates an already accentuated edematous condition in such affected women. Since glucocorticoids cause virtually no retention of sodium, they should be of particular value in treating women affected with chronic hereditary lymphedema.

SUMMARY

Genetic and physiological studies have been made on a family in which twelve individuals (six males, six females) are affected with chronic hereditary lymphedema (Milroy's disease) of the tardive type. This condition has been traced through four generations of the family and is inherited as a dominant autosomal trait probably with complete penetrance. The age at onset varies from eleven years to forty-two years.

Six affected members and seven unaffected relatives or spouses of the affected individuals were given citrus bioflavonoid-vitamin C compound (600 mg. of each daily in divided doses) for a thirty day period. Measurements were taken of the lower extremities and relatively little variation was found in the controls. Considerable daily variation in the size of the lower extremities was recorded by affected individuals, and a downward trend was observed in three persons. Studies will be continued for a further evaluation of CVP therapy, and also glucocorticoid therapy in chronic hereditary lymphedema.

One individual inherited chronic hereditary lymphedema from his father and angioneurotic edema from his mother. Apparently, there is independent action of the two genes responsible for these traits. Angioneurotic edema has been reported in eight individuals (four males, four females) in four generations of this second family and has been inherited as an autosomal dominant trait with complete penetrance. Two individuals have died as a direct result of angioneurotic edema of the glottis.

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The ABO Blood Groups and Masculinity of Offspring at Birth

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IN 1951 SANGHVI pointed out discrepancies in the masculinity* of infants in certain mother-child ABO blood group combinations. Sanghvi's observations were based on two samples, one of 1,330 maternity cases in a hospital in Bombay, the other of 865 maternity cases, mainly Rh-negative women in a hospital in New York. In both these samples there was a significantly higher masculinity for O infants born to O mothers when compared directly with A infants born to A mothers. In an attempt to confirm Sanghvi's observations Johnstone (1954) tabulated the results of 2,429 sex and blood group determinations for infants born in a London hospital. Johnstone's figures do not show a significant difference between the O-O and A-A combinations, though the O-O combination does have a higher masculinity than the A-A combination. Various aspects of Sanghvi's and Johnstone's data have been discussed also by Allen (1952; 1953; and 1954).

More recently Cohen and Glass (1956) have added two further samples, one of women attending the Baltimore Rh typing laboratory, and the other comprising mothers and offspring (not newborn infants) involved in disputed paternity cases. In both these samples negro and white women are included.

Cohen and Glass have pooled their own samples with those of Sanghvi and Johnstone, giving a total sample of 9,162 cases. Individual contributions to χ^2 for the fourteen possible ABO mother-child combinations shows that two of them, the A-A and B-B combinations differ significantly from expectation based on the masculinity of the whole sample. The authors point out that their own samples and those of Sanghvi and Johnstone consistently show a lower masculinity for the A-A combination and a higher masculinity for the B-B combination than the masculinity of the whole samples. These differences contribute largely to an apparent low masculinity for the offspring of all group A mothers and the group A offspring of all mothers and a high masculinity of the group B offspring of all mothers.

Cohen and Glass discuss certain possible mechanisms which might explain these observed deviations in masculinity. Some of the possibilities are rejected because of contradictory evidence in their own material, but they leave one hypothesis for future testing. They suggest that the low or high masculinities occur in those matings where O- bearing eggs in females of types A or B are fertilized by spermatozoa carrying A or B respectively.

During the last few years we have had the opportunity of examining the records of a large number of cases in two Australian maternity hospitals. Our series now

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* We have used the term masculinity throughout this paper. It refers to the number of males per 100 infants at birth.

comprises over 15,000 deliveries where adequate blood group information on both mother and newborn infant is available. This is more than three times the size of the largest single sample used by the workers cited above, and considerably larger than the total pooled sample analysed by Cohen and Glass. Though we are now collecting such information as a routine at the rate of approximately 7,000 new cases a year, we think it worthwhile presenting our analysis at this stage since although it shows some similarities with the pooled data of Cohen and Glass, it disagrees in certain important respects with the findings in their material.

MATERIAL AND METHODS

The records used here have been obtained from two sources:

(a) All deliveries in the Queen Victoria Hospital, Melbourne during the years 1953, 1954 and 1955, and (b) all deliveries in the King Edward Memorial Hospital for Women, Perth during the years 1955 and 1956.

In both samples several hundred records were rejected because of insufficient information on the maternal blood group. There is no reason to believe that any bias operated in the non-availability of the mother's blood group. A frequent reason for lack of information about the infant's blood group in the small number of cases where it was not obtained was the failure to collect cord-blood samples in some cases of stillbirth. This would bias the remaining records if fetal death was associated more commonly with one or more blood groups, as would be the case in severe cases of ABO hemolytic disease of the newborn. A small number of records was rejected because of impossible mother-child combinations. There were 4 such combinations in the Melbourne data and 2 in the Perth data.

Multiple births have been excluded also. The two samples, therefore relate to 11,508 single-birth deliveries in Melbourne and 3,967 single-birth deliveries in Perth. In both places some of the mothers will be entered more than once in the sample if they had a second child during the period under review.

The procedures for typing mothers and infants used in Melbourne have been given by Bryce, Jakobowicz, McArthur and Penrose (1950). The methods of typing mothers and infants in Perth are given by Kirk & Vos (1957). In the latter paper an attempt has been made to assess the accuracy of routine cord-blood ABO typing by testing the A and B secretor status of all infants born. No misgrouping has been detected in over 500 tests carried out.

RESULTS

The number of offspring and the masculinity in each of the fourteen possible mother-child ABO blood group combinations is given in table 1 for the 11,508 single births in Melbourne and the 3,967 single births in Perth. Since a comparison of the two distributions shows them to be homogenous ($\chi^2_{(13)} = 16.19; 0.30 > P > 0.20$) table 1 includes also the same information for the Melbourne and Perth samples combined. The $\chi^2_{(1)}$ values in table 1 were obtained in the usual way from the differences between the observed and expected number of males and females for each mother-child combination; the expected numbers were based on the proportion of males and females in the whole sample.

TABLE 1. NUMBER OF OFFSPRING, MASCULINITY AND CONTRIBUTION TO TOTAL χ^2 BY ABO MOTHER-CHILD COMBINATIONS

Blood Group Combination..... Mother-child	Melbourne Sample			Perth Sample			Melbourne and Perth		
	No.	Masculinity	χ^2	No.	Masculinity	χ^2	No.	Masculinity	χ^2
O-O	3,735	53.33	0.47	1,261	54.72	1.15	4,996	53.68	1.28
O-A	1,178	51.02	1.46	436	51.38	0.59	1,614	51.12	2.04
O-B	366	49.73	1.35	118	53.39	0.00	484	50.62	1.00
A-O	1,358	54.27	1.23	446	55.16	0.68	1,804	54.49	1.86
A-A	2,950	51.90	0.91	1,015	51.53	1.16	3,965	51.80	1.84
A-B	161	50.93	0.22	68	51.47	0.09	229	51.09	0.30
A-AB	138	62.32	5.06	59	55.93	0.18	197	60.41	4.46
B-O	386	50.00	1.19	146	47.95	1.63	532	49.44	2.53
B-A	146	42.47	6.18	52	46.15	1.05	198	43.43	7.09
B-B	537	54.38	0.55	163	49.69	0.80	700	53.29	0.05
B-AB	133	56.39	0.70	61	55.74	0.15	194	56.19	0.85
AB-A	215	57.21	1.68	67	67.16	5.18	282	59.57	5.09
AB-B	156	57.05	1.15	51	60.78	1.20	207	57.97	2.14
AB-AB	49	57.14	0.36	24	50.00	0.11	73	54.79	0.11
All Groups	11,508	52.77		3,967	53.21		15,475	52.89	
Total χ^2			22.51			13.97			30.64

In the two individual samples and in the combined sample the individual contributions to χ^2 have been calculated for each mother-child combination to give a probability estimate for the deviation of the observed number of males and females in each cell from the sample masculinity. In neither the Melbourne sample or the Perth sample is it likely, since the sum of the χ^2 does not exceed the conventional level of significance, that the observed distribution of male and female offspring is due to factors other than chance. (For the Melbourne sample $\chi^2_{(13)} = 22.5$; $P = 0.05$ and for Perth $\chi^2_{(13)} = 14.0$; $0.50 > P > 0.30$). When both samples are combined, however, the probability of getting such a distribution is less than 1 in a 100. ($\chi^2_{(13)} = 30.6$; $P < 0.01$).

For the Melbourne sample two of the mother-child combinations show significant deviations from the mean at the 5 per cent level. The A children of B mothers have a significantly low masculinity, while the AB children of A mothers have a significantly high masculinity. Only the A children of AB mothers show a deviation significant at the 5 per cent level in the Perth sample, in this case a higher masculinity. This deviation is somewhat reduced in the combined sample, but the high masculinity of AB children of A mothers is also significant at the 5 per cent level in the combined sample and the low masculinity of the A children of B mothers is significant at the 1 per cent level.

These figures do not lend weight to the hypothesis that factors operating in the ABO blood group system influence the masculinity of newborn infants. In any series of 14 samples chosen at random from a population it is not unlikely that one or even two samples will deviate from expectation at the 5 per cent level, and there is also approximately 1 chance in 7 that one of the samples will deviate from expectation at the 1 per cent level. The two combinations with the largest number of cases, i.e. group O children of O mothers and group A children of A mothers, though

TABLE 2. THE NUMBER OF MALES AND FEMALES IN ABO-COMPATIBLE AND INCOMPATIBLE MOTHER-CHILD COMBINATIONS

	Compatible			Incompatible			
	Males	Females	Masculinity	Males	Females	Masculinity	χ_1^2
Melbourne	4,985	4,401	53.11	1,088	1,034	51.27	2.34
Perth	1,698	1,475	53.51	413	381	52.02	0.57
Melbourne & Perth	6,683	5,876	53.21	1,501	1,415	51.47	2.87

they deviate from the mean in the same direction as that reported originally by Sanghvi, do not do so significantly either in the Melbourne, Perth or combined samples.

Since we have used similar mother-child samples to evaluate the possibility of selection due to incompatibility in the ABO blood group system (Kirk *et al* 1955) and since infants dying from erythroblastosis have a masculinity of approximately 0.63 (based on the mean for England and Wales 1932–1949 and Australia 1939–1950) it seemed reasonable to test the hypothesis that infants in incompatible mother-child combinations have a lower masculinity than those in compatible combinations. Table 2 shows that this is true in both the Melbourne and Perth samples. The difference however, is not significant in the individual samples or in the combined sample.

From recent clinical studies (Zuelzer and Kaplan, 1954; Rosenfield, 1955; Reepmaker and Van Loghem, 1956; and others) it seems that erythroblastosis due to ABO incompatibility is confined almost entirely to the A or B children of group O mothers. We have tested the possibility therefore that the masculinity of offspring in O-A and O-B combinations together is different from the masculinity of all other mother-child combinations. Again, although A and B children of O mothers together have a lower masculinity than children in all other combinations, the difference is not significant.

Finally, table 3 gives the number of infants, masculinity and chi-square values by blood group of the mother, and also by blood group of the child. In the combined sample the infants of AB mothers have a significantly high masculinity ($P < 0.01$). There are no outstanding variations in the masculinity of infants belonging to different blood groups. In the Melbourne and the combined samples the deviations of AB infants just reach the 5 per cent level of significance.

It is of interest to note that 12.65 of the total chi-square of 30.64 which suggests that there is a significant degree of heterogeneity in the combined Melbourne and Perth sample in table 1 is contributed by the offspring of AB mothers or the AB offspring of all mothers. These two categories are subject to the greatest percentage error in routine blood grouping procedures, and it is possible that this may explain some of the heterogeneity in masculinity observed in these particular mother-child combinations. In an attempt to substantiate the significantly high masculinity of infants of AB mothers in the combined sample the sex of infants born to all AB mothers in the King Edward Memorial Hospital during the years 1948–1952 has been recorded. There were 233 deliveries to AB mothers during this period and the masculinity was 47.64. This result suggests that a more careful study of the mascu-

TABLE 3. THE NUMBER OF INFANTS, MASCULINITY AND χ^2 BY BLOOD GROUP OF MOTHER AND BY BLOOD GROUP OF CHILD

	O			A			B			AB		
	No.	Masculinity	χ_1^2	No.	Masculinity	χ_1^2	No.	Masculinity	χ_1^2	No.	Masculinity	χ_1^2
Mothers												
Melbourne	5,279	52.57	0.09	4,607	52.88	0.02	1,202	51.75	0.51	420	57.14	3.22
Perth	1,815	53.83	0.28	1,588	52.71	0.17	422	49.53	2.31	142	61.97	4.35
Melbourne & Perth	7,094	52.89	0.00	6,195	52.83	0.01	1,624	51.17	1.92	562	58.36	6.77
Children												
Melbourne	5,479	53.33	0.68	4,489	51.62	2.41	1,220	52.87	0.01	320	59.06	5.09
Perth	1,853	54.29	0.86	1,570	51.97	0.98	400	52.50	0.08	144	54.86	0.17
Melbourne & Perth	7,332	53.57	1.39	6,059	51.71	3.37	1,620	52.78	0.01	464	57.76	4.42

linity of offspring of AB mothers will be needed before any certainty about a significant departure from the masculinity of the whole sample can be accepted.

DISCUSSION

The analysis of the pooled samples of Sanghvi, Johnstone and their own material from Baltimore by Cohen and Glass yielded a highly significant low masculinity for A children of A mothers and a significantly high masculinity for the B children of B mothers. On a much larger and more homogeneous sample our own figures do not reveal any departure from expectation in these two categories ($\chi^2_{(1)} = 1.84$; $0.2 > P > 0.1$, and $\chi^2_{(1)} = 0.05$; $0.9 > P > 0.8$ respectively.) Similarly there is no indication in our own data that the proportion of males and females in the matching combinations O-O, A-A, B-B and AB-AB are heterogeneous ($\chi^2_{(3)} = 3.28$; $0.5 > P > 0.30$) though in the pooled sample of Cohen and Glass the probability that infants in these matching combinations are from a homogeneous population is less than 0.001. Nor is there any indication in our own combined sample that the masculinity of offspring is significantly different from expectation by blood group of the mother or by blood group of the child, except for the offspring of group AB mothers. The high masculinity of these infants is significant at the 1 percent level.

It might be claimed that even though our own figures do not reveal a significantly low masculinity for children in the A-A combination and a significantly high one for children in the B-B combination, they do strengthen the argument advanced by Cohen and Glass since in fact the masculinity of A infants from A mothers in our sample is lower and the masculinity of B infants from B mothers is higher than the sample masculinity. In both of Sanghvi's samples, in that of Johnstone, in the multiple pooled Baltimore sample and the combined Melbourne and Perth sample there is consistency: in all these samples the A-A combination has a lower and the B-B combination a higher masculinity than the corresponding sample masculinity. (It should be noted, however, that the Melbourne and Perth samples separately are

TABLE 4. POSITIVE OR NEGATIVE DEVIATIONS BETWEEN MASCULINITY OF OFFSPRING FOR MOTHER-CHILD COMBINATIONS, AND THE MEAN OF THE SAME SAMPLE

Sample	Child Group	Mother Group O	A	B	AB
B. P. M.		+	+	+*	
B		+	−	−*	
N. Y.	O	+	−	−	
L		−	+	+	
M. P.		+	+	−	
B. P. M.		+	−	+	−
B		−	−	+	−
N. Y.	A	−	−	−	+
L		+	−	+	+
M. P.		−	−	−	+
B. P. M.		+	−	+	+
B		+	−	+	+
N. Y.	B	−	+	+	+
L		+	+	+	−
M. P.		−	−	+	+
B. P. M.			+	−	+
B			−	−	+
N. Y.	AB		+	−	+
L			−	−	−
M. P.			+	+	+

B. P. M: Boston Pooled Multiple (Cohen and Glass: 1956)
B: Bombay (Sanghvi: 1953)
N. Y. New York (Sanghvi: 1953)
L: London (Johnstone: 1954)
M. P. Melbourne & Perth (Present series)
* Note: To the first decimal these differences were zero. One has been assigned +, the other −

TABLE 5. DISTRIBUTION OF EXPECTED AND OBSERVED SIGN OF DEVIATIONS RECORDED IN TABLE 4

	All + or All −	4 + or − / 1 − or +	3 + or − / 2 − or +	Total
Expected	0.875	4.375	8.750	14
Observed	2	4	8	14

inconsistent for the B-B combination.) In table 4 the positive or negative deviations of the masculinity in each mother-child combination from the corresponding sample mean are given for the five samples listed above. If it is assumed that the chance of getting a positive or negative deviation is equal, the distribution of expected sequences of positive and negative values is obtained simply from the terms of the binomial expansion of $(\frac{1}{2}a + \frac{1}{2}b)^5$ when a is a positive and b is a negative deviation. The expected and observed number in the different classes are given in table 5. The two distributions are almost identical. The fact, therefore, that in a series of five independent samples out of the fourteen possible mother-child combinations two of the combinations show consistency in the sign of the deviation of masculinity from the sample masculinity does not mean that undue weight should be given to this consistency. If an *a priori* hypothesis had predicted that such deviations should

occur in these two combinations, then the consistency would have much greater significance.

We must draw attention also to the fact that the very high values for chi-square for the deviation in the number of males and females in the A-A and B-B combinations in Cohen and Glass's paper is based on pooling very dissimilar samples. The authors state that "statistical analysis having shown that the Baltimore series does not differ significantly from the pooled New York and Bombay series of Sanghvi or of the London series of Johnstone, these have all been combined in one pooled B-S-J sample ($\chi^2_{(28)} = 25.44; 0.70 > P > 0.50$)". The samples are, however, heterogeneous in several ways not revealed by their method of analysis. The two samples of Sanghvi are heterogeneous in blood group distribution, and Sanghvi refrained from pooling them in his original paper (Sanghvi 1953). His New York sample unlike his Bombay sample is based mainly on Rh-negative women, as is the largest part of Cohen & Glass's sample in Baltimore. The latter sample includes blood group determinations on children at times other than at birth, and also both negro and white women. All the samples almost certainly differ in the mean number of children per woman. Johnstone's sample is for a single ascertainment per woman, whilst this is not true for the Baltimore sample and the position is uncertain in Sanghvi's samples. These differences could easily contribute fortuitously to an apparent heterogeneity in the number of male and female offspring in some of the mother-child combinations in the total pooled sample. In a recent paper Edwards (1957) has drawn attention also to the difficulties which arise from pooling these very dissimilar samples. Since the heterogeneity present in the A-A and B-B combinations of the pooled data analysed by Cohen and Glass is not present in these combinations in our own much larger and more homogeneous sample we believe this is the more likely explanation of their results.

In the absence of any significant deviation for infants in the A-A and B-B combinations in our own samples our figures lend no support to the ingenious hypothesis put forward by Cohen & Glass to explain these deviations in their own pooled sample. Further, there is at the present time no explanation of why a group A X-bearing sperm is more likely to fertilize a group O egg in a group A mother than a group A Y-bearing sperm, or alternatively why such an egg fertilized by a group A Y-bearing sperm is more likely to die than one fertilized by a group A X-bearing sperm.

On the other hand, a more likely hypothesis is that incompatible anti-A or anti-B antibodies crossing the placenta into the fetus selectively eliminate more males than females in early uterine life. But although the deviations in masculinity between the compatible and incompatible ABO blood group combinations are in the expected direction in both the individual samples in our material the deviations are non-significant. This hypothesis is not supported either by the results published by Sanghvi, Johnstone, or by Cohen and Glass.

It is of interest to calculate what size of sample would be necessary to demonstrate a lowering of the masculinity in the most likely incompatible combinations, i.e. O mother $-$ A or $-$ B infant.

If A is the total number of A and B infants conceived by O mothers, and S is their viability up to the time of birth, then the number of fetuses lost will be A(1

– S). The expected number of males among these will be MA (1 – S) where M is the masculinity of all births. If, however, the selective process is eliminating more males than females, so that the masculinity of the fetuses dying is M_d, the actual number of males among the fetuses lost will be M_d A(1 – S). The difference between the expected and observed number of A and B males born to group O mothers, therefore, will be A(1 – S) (M – M_d).

If selection is operating, but with no preference for the sex of the fetuses eliminated, i.e. if M_d = M, the actual number of males in these categories would be MSA, and the corresponding number of females would be (1 – M)SA. The difference between the observed and expected number of male and female A and B infants born to O mothers can now be used to calculate χ_1^2 in the usual manner, so that

$$\chi^2_{(1)} = A\left[\frac{(1-S)(M-M_d)}{MS}\right]^2 + A\left[\frac{(1-S)(M-M_d)}{(1-M)S}\right]^2$$

Using the most probable value of S = 0.93 (Reed, 1956, based on our previous work Kirk, *et al*, 1955), and assigning a value of 0.66 to M_d (i.e. twice the number of males to females eliminated) and using a value of 0.51 for M (close to the Australian mean) the value of A becomes 23,000 for $\chi^2_{(1)}$ = 10.8. This would give a probability of 1 in 1,000 for the deviation in the observed number of male and female A or B infants born to group O mothers.

Since the number of A and B infants to group O mothers in our Australian sample is approximately one-seventh of the total number of children born to all mothers, a total sample of 160,000 births would need to be examined to establish beyond reasonable doubt the significance of the deviation in the number of males and females among group A and group B children of O mothers if the assumptions first made actually hold.

From the above it will be seen that with χ^2 constant A varies inversely as $\frac{(1-S^2)}{S}$ and also inversely as $(M - M_d)^2$. If therefore we substitute a value of S = 0.97, instead of 0.93, and M_d = 0.58 instead of 0.66 the sample size required to give the same degree of probability to the deviation in number of males and females in the categories under examination would be very much larger than that based on the assumptions used in the previous calculation. A total sample of 4,000,000 births would need to be analysed. Since both these values are plausible it is obvious that very large samples would be needed to verify beyond reasonable doubt that the observed deviations are not due to chance.

The result of the present analysis indicates, therefore, that speculation at present on the relationship between the ABO blood groups and masculinity of offspring at birth is premature. The existing studies are based on small samples, the largest being the Melbourne and Perth sample of the present paper. Several mother-child combinations give deviations from the sample masculinity significant at either the 5 percent or 1 percent level. However, it is clear that where a large number of comparisons is being made, as in the present instance, random deviations significant at the 5 percent level are not important, and even at the 1 percent level they suggest only that further investigation is needed.

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SUMMARY

A statistical analysis of 11,508 single births in Melbourne, and 3,967 single births in Perth has been carried out to see if there is any association between the number of males and females born and the ABO blood group status of mother and infant.

There is no significant heterogeneity in the proportion of males and females in either Melbourne or Perth samples. In the combined Melbourne and Perth sample the total chi-square reaches the 1 percent level of significance. The main contribution to this total is from the combinations of infants from AB mothers, and AB infants from all mothers. Further study is needed to demonstrate the validity of the deviations in these categories.

In individual mother-child blood group combinations there is a high masculinity of AB children to A mothers in the combined Melbourne and Perth sample which is significant at the 5 percent level and there is a low masculinity of A children to B mothers in the combined sample significant at the 1 percent level. It is pointed out that some deviations at this level of significance are likely to occur by chance in any series of 14 random samples.

The results reported here do not support the hypotheses put forward by other workers. It is pointed out also that a test of the possible disturbance of the proportion of males to females through selection of incompatible A or B fetuses in group O mothers would require a sample of at least 160,000 births to achieve significance at the .001 level.

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Blood Donor Controls for Blood Group Disease Researches

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INTRODUCTION

SINCE THE REPORT BY AIRD *ET AL* (1953), increasing numbers of communications have appeared suggesting an association between the ABO blood groups and certain diseases. The evidence for such associations is the finding of statistically significant differences between the blood type frequencies of patients, and those of "healthy" persons (controls). Blood donors have most often been used as the controls. It has been assumed that blood donors provide reliable "normal" blood type frequencies as they are "healthy" persons not having the disorder being studied.

In our investigations, Buckwalter *et al*, (1956), the controls were random samples from consecutive voluntary blood donors seen at the Methodist Hospital, Des Moines and the University Hospitals during 1952 through 1954 (Controls I). The blood type frequencies agreed closely with those quoted for Iowa and the United States, with those observed in a University Hospital patient group (excluding patients with gastric carcinoma and duodenal ulcer), and with those of blood donors seen at other Iowa hospitals. It was therefore assumed that a satisfactory control sample had been obtained. However, as our investigations progressed, questions arose indicating a need for a more exhaustive study of the blood donor controls. Some of the questions were: (1) Was the number of blood donors large enough to provide statistically satisfactory control data? (2) Were the donors seen during three years representative of the population which provided patients during a 17 year period? (3) Were we correct in assuming that the "normal" blood type frequency of the population does not change with age? (4) Were the blood type frequencies of the sexes the same; if so, does it remain so at different ages? (5) Did unsuspected factors such as the season influence the observed blood type frequencies? (6) What effect did inadvertent inclusion of professional donors have? (7) Are blood donors reliable controls for these investigations? Are there better controls? The results of the investigations designed to answer these questions follow.

MATERIALS AND METHODS

Data were obtained from the records of the University Hospitals' blood bank, 1940–1956 (Controls II). All the blood typing during the 17 years was performed by

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blood bank personnel under the supervision of Dr. E. L. DeGowin who established the blood bank in 1938. A slide technique has been employed. The only significant technical change during this interval was the use of progressively higher titer antisera.

Only voluntary blood donors, friends or relatives of the patients who came to the University Hospital to replace blood given, were included. No donors were selected because of their blood type. The only prospective donors rejected were those failing to meet health requirements. Professional donors were excluded except as stated. The race of the blood donors was not recorded. About two per cent of the donors and about two per cent of the patients were Negroes. The number of donors or patients of other non-white racial groups was negligible. No attempt was made to exclude the infrequent repetitious voluntary donor because there is no apparent cause for a higher incidence of repetitious donors of one blood type than another.

The age, sex, and ABO blood type of consecutive voluntary donors during January, February, May, June, July, August, November and December of each year were recorded. The number of voluntary blood donors in each year is given in table 3.

The statistical significance of the observed differences in blood type frequencies between the several years, was determined by the Chi Square (χ^2) method. Differences occurring in the O:A frequencies, and those occurring simultaneously for the four blood types, O:A:B:AB, were examined. This method of examining the data was described in detail in an earlier communication, Buckwalter *et al* (1956).

RESULTS AND DISCUSSION

The original control data (Controls I), and the later control data (Controls II) with which this report is primarily concerned, are recorded in table 1. Notice that the differences considering, (1) only those of blood types O and A, and (2) those occurring simultaneously in the four blood types, are not statistically significant. Of interest is the higher frequency of blood type O, lower frequency of blood type A, in the original controls. As noted before, Controls I consisted of random samples of blood donors from the years 1952 through 1954. In contrast, Controls II consist of about three thousand donors from each of 17 years, 1940 through 1956. Possibly, the original "random" sample selected higher O and lower A frequency donors. More care was exercised in excluding professional donors from Controls II than from Controls I. The effect of including professional donors is shown in table 2. These data were obtained from blood donors (voluntary and professional) seen during July and August of each of the 17 years. They reflect the clinical needs dictated by the relatively high demand for blood type O (universal donor blood). Notice that the inclusion of professional donors raised the frequency of blood type O at the expense of the three other blood types. The differences between the two groups of data, including and excluding the professional donors, are statistically significant.

The blood type data for each of the 17 years are recorded in table 3. A Chi Square test of the data given in table 3 resulted in $\chi^2 = 86.66$ with 48 degrees of freedom; a result which is significant at the 1 per cent level. Further investigation revealed that the greatest differences of the observed from the expected occurred during 1951 (AB blood type), 1941 (B blood type), and 1954 (AB blood type), with somewhat

TABLE 1. COMPARISON OF BLOOD TYPE FREQUENCIES OF CONTROLS I (1952–54 BLOOD DONORS) AND CONTROLS II. WHEN THE 4,690 UNIVERSITY HOSPITAL 1952–54 BLOOD DONORS COMMON TO CONTROLS I AND CONTROLS II ARE EXCLUDED, $P > .10$ FOR O:A AND O:A:B:AB COMPARISONS.

Controls		Blood Type				Total	O:A		O:A:B:AB	
		O	A	B	AB		χ^2	Prob.	χ^2	Prob.
I	No.	2,892	2,625	570	226	6,313				
	%	45.8	41.6	9.0	3.6	100.0				
							1.908	$P > .10$	2.848	$P > .10$
II	No.	22,392	21,144	4,695	1,748	49,979				
	%	44.8	42.3	9.4	3.5	100.0				

TABLE 2. EFFECT OF PROFESSIONAL DONORS ON BLOOD TYPE FREQUENCIES. NOTE THE HIGHLY SIGNIFICANT DIFFERENCES BETWEEN THE PROFESSIONAL AND VOLUNTARY DONORS ARE REFLECTED IN SIGNIFICANT DIFFERENCES OBSERVED BETWEEN TWO BLOOD DONOR SAMPLES (WITH AND WITHOUT PROFESSIONAL DONORS).

Blood Donors		Blood Type				Total Donors	Statistical Analysis			
							O:A		O:A:B:AB	
		O	A	B	AB		χ^2	Prob.	χ^2	Prob.
Professional	No.	2,609	2,075	446	157	5,287				
	%	49.35	39.25	8.43	2.97					
							29.41	.001 > P	36.38	.001 > P
Voluntary	No.	5,462	5,257	1,168	408	12,295				
	%	44.42	42.76	9.50	3.32					
							5.27	.05 > P > .02	6.45	.10 > P > .05
Professional and Voluntary	No.	8,071	7,332	1,614	565	17,582				
	%	45.90	41.70	9.18	3.21					

smaller differences during 1947 (O and A blood types). A supplementary investigation of the data during these years indicated that the differences referred to above were somewhat inflated; for example, in a second sample of 1001 in 1941 the per cent in the B blood type was 8.49 instead of 7.79 as given in table 3; in a second sample of 1001 in 1951 the per cent in the AB blood type was 2.70 instead of 2.49 as given in table 3; and in a second sample of 1028 in 1954 the per cent in the AB blood type was 3.79 instead of 4.41 as given in table 3. A second sample of 1026 in 1947 revealed a similar situation with respect to the O and A blood types (47.27 compared to 47.35 and 40.16 compared to 39.76). It is interesting to observe that in each of the second samples, including 1947, the percentages approach the percentages given in the subtotal, table 3. No significant difference was found in the data which make up the subtotal.

No satisfactory explanation for the findings in 1941 (type B), 1951 (type AB), and 1954 (type AB) which are responsible for the heterogeneity of this data can be suggested. As indicated above, when these three years are excluded, the data appear homogeneous. Note the close agreement of the blood type frequencies recorded in the subtotal and total.

TABLE 3. NUMBER AND PERCENTAGE OF VOLUNTARY BLOOD DONORS OF EACH ABO BLOOD TYPE, UNIVERSITY HOSPITALS BLOOD BANK, 1940 THROUGH 1956

Year	Blood Type								Total No. Donors
	O		A		B		AB		
	No.	%	No.	%	No.	%	No.	%	
1940	938	43.79	898	41.92	227	10.60	79	3.69	2,142
1941	1,254	46.76	1,142	42.58	209	7.79	77	2.87	2,682
1942	1,279	43.76	1,265	43.28	280	9.58	99	3.39	2,923
1943	1,357	44.51	1,310	42.96	280	9.18	102	3.35	3,049
1944	1,462	45.08	1,384	42.68	280	8.63	117	3.61	3,243
1945	1,367	44.11	1,317	42.50	304	9.81	111	3.58	3,099
1946	1,420	43.39	1,438	43.94	299	9.13	116	3.54	3,273
1947	1,484	47.35	1,246	39.76	288	9.19	116	3.70	3,134
1948	1,313	42.89	1,323	43.22	306	10.00	119	3.89	3,061
1949	1,435	45.61	1,280	40.69	313	9.95	118	3.75	3,146
1950	1,515	47.05	1,343	41.71	267	8.29	95	2.95	3,220
1951	1,337	44.45	1,321	43.92	275	9.14	75	2.49	3,008
1952	1,234	44.87	1,146	41.67	273	9.93	97	3.53	2,754
1953	1,242	45.73	1,116	41.09	266	9.79	92	3.39	2,716
1954	1,276	42.62	1,281	42.78	305	10.19	132	4.41	2,994
1955	1,284	45.10	1,204	42.29	267	9.38	92	3.23	2,847
1956	1,195	44.39	1,130	41.98	256	9.51	111	4.12	2,692
Sub Total*	18,525	44.86	17,400	42.14	3,906	9.46	1,464	3.55	41,295
Total	22,392	44.80	21,144	42.31	4,695	9.39	1,748	3.50	49,979

* Excludes 1941, 1951, and 1954.

TABLE 4. COMPARISON OF THE BLOOD TYPE FREQUENCIES OF 1940–1948 AND 1949–1956 BLOOD DONORS

Year		Blood Type				Total	O:A		O:A:B:AB	
		O	A	B	AB		χ^2	Prob.	χ^2	Prob.
1940–1948	No.	11,874	11,323	2,473	936	26,606				
	%	44.63	42.56	9.29	3.52	100.00				
							1.199	P > .10	1.868	P > .10
1949–1956	No.	10,518	9,821	2,222	812	23,373				
	%	45.00	42.02	9.51	3.47	100.00				

The use of higher titer antisera might be expected to lower the blood type O frequency. Note that there is a slight increase in blood type O frequency (the difference is not statistically significant) when the data from the first and last half of the 17 years are compared (table 4). The higher titer antisera may not have resulted in the expected detection of more persons with blood types A, B, and AB and fewer with type O, or, more A, B, and AB, and fewer O blood types may have been detected, but this was offset by a simultaneous increase in blood type O frequency in the population during the later years. The answer to this question is not provided by the data; the first explanation seems more plausible.

TABLE 5. NUMBER AND PERCENTAGE OF VOLUNTARY BLOOD DONORS OF EACH ABO BLOOD TYPE, BY AGE GROUP AND SEX

Age in Years	Sex	Blood Type								Total No. Donors
		O		A		B		AB		
		No.	%	No.	%	No.	%	No.	%	
	Males	1,959	43.04	1,998	43.89	432	9.49	163	3.58	4,552
<25	Females	964	46.32	846	40.65	197	9.47	74	3.56	2,081
	Sex Unknown	2,042	44.30	1,941	42.11	453	9.83	173	3.75	4,609
	Males	1,648	44.50	1,618	43.69	307	8.29	130	3.51	3,703
25–29	Females	601	44.45	582	43.05	125	9.25	44	3.25	1,352
	Sex Unknown	1,532	45.05	1,433	42.13	334	9.82	102	3.00	3,401
<30	Total	8,746	44.40	8,418	42.74	1,848	9.38	686	3.48	19,698
	Males	1,615	45.42	1,479	41.59	345	9.70	117	3.29	3,556
30–34	Females	599	46.08	545	41.92	108	8.31	48	3.69	1,300
	Sex Unknown	1,474	47.17	1,252	40.06	281	8.99	118	3.78	3,125
	Males	1,433	43.88	1,414	43.29	311	9.52	108	3.31	3,266
35–39	Females	610	45.80	539	40.46	124	9.31	59	4.43	1,332
	Sex Unknown	1,353	45.10	1,265	42.17	274	9.13	108	3.60	3,000
30–39	Total	7,084	45.47	6,494	41.69	1,443	9.26	558	3.58	15,579
	Males	1,191	45.18	1,086	41.20	254	9.64	105	3.98	2,636
40–44	Females	474	41.98	523	46.32	96	8.50	36	3.19	1,129
	Sex Unknown	947	43.94	912	42.32	208	9.65	88	4.08	2,155
	Males	840	45.65	775	42.12	171	9.29	54	2.93	1,840
45–49	Females	366	44.91	346	42.45	77	9.45	26	3.19	815
	Sex Unknown	797	44.95	757	42.70	156	8.80	63	3.55	1,773
40–49	Total	4,615	44.60	4,399	42.51	962	9.30	372	3.59	10,348
	Males	499	42.65	510	43.59	131	11.20	30	2.56	1,170
50–54	Females	246	41.77	263	44.65	59	10.02	21	3.56	589
	Sex Unknown	425	45.45	388	41.50	90	9.63	32	3.42	935
	Males	353	48.49	281	38.60	73	10.03	21	2.88	728
>54	Females	137	45.21	124	40.92	33	10.89	9	2.97	303
	Sex Unknown	287	45.63	267	42.45	56	8.90	19	3.02	629
>49	Total	1,947	44.72	1,833	42.10	442	10.15	132	3.03	4,354
Totals	Males	9,538	44.46	9,161	42.71	2,024	9.44	728	3.39	21,451
	Females	3,997	44.91	3,768	42.33	819	9.20	317	3.56	8,901
	Sex Unknown	8,857	45.13	8,215	41.86	1,852	9.43	703	3.58	19,627
Grand Total		22,392	44.80	21,144	42.31	4,695	9.39	1,748	3.50	49,979

TABLE 6. COMPARISON OF BLOOD TYPE FREQUENCIES OF DONORS GIVING BLOOD DURING WINTER AND SUMMER

Season		Blood Type				Total Donors	Statistical Analysis			
							O:A		O:A:B:AB	
		O	A	B	AB		χ^2	Prob.	χ^3	Prob.
Winter	No.	3,751	3,582	832	335	8,500				
	%	44.13	42.14	9.79	3.94					
							.06	P > .10	4.83	P > .10
Summer	No.	3,777	3,635	800	288	8,500				
	%	44.43	42.77	9.41	3.39					

Any shift in the blood type frequencies related to ageing, would have important implications. A higher incidence of certain diseases in persons of a specific blood type would act to reduce the number of those with this blood type in the older age groups. For example, carcinoma of the stomach, a highly lethal disease with a significantly higher incidence in persons with blood type A, would cause a reduction in the frequency of this blood type with advancing age. However, if, as seems possible and perhaps probable, equally lethal diseases have an increased incidence in persons of the other blood types, the effect of carcinoma of the stomach and other diseases might balance one another. Thus diseases may act as dynamic selective factors helping to determine the blood type frequencies of any population. Reduction in fertility rates related to disease contracted before or during the reproductive years, affects blood type frequencies by reducing the expected number of progeny of these patients. In carcinoma of the stomach this effect is minimal as compared with peptic ulcer which occurs in higher incidence at an earlier age. It is apparent that knowlege of the blood type frequencies of the "healthy" population at different ages is essential if valid conclusions are to be drawn from comparisons of blood type frequencies of blood donors from such a population, and patients with diseases having varying age incidences

The blood types of the donors by sex and age groups are recorded in table 5. No statistically significant differences in the blood type frequencies between the various age groups were found. This was true when the data were divided into four groups (10 year intervals: 4 x 4 table, d. f. = 9 χ^2 = 11.06) or eight groups (5 year intervals: 8 x 4 table, d. f. = 21 χ^2 = 27.63). Since most of the blood donors were from younger age groups, the older age groups are smaller in numbers and therefore, are of less significance. More data for older persons of the "healthy" population will be necessary to provide a convincing answer to this question.

As anticipated, the blood type frequencies of men and women did not differ significantly. The possibility that the blood type frequencies of the sexes might differ by age groups was examined and no differences of significance were found (table 5).

There may be other unknown factors acting to influence the blood type frequency of the blood donors. Season of the year when the blood was obtained might be one. Farmers make up a higher proportion of the blood donors in the winter than summer. An excess or dearth of such donors, strong vigorous men, might influence the blood

type frequencies of the blood donors. The data from winter and summer donors seen during the 17 year period are recorded in table 6. No evidence of heterogeneity between the two groups of data is observed. It is essential that investigators continue to search for such factors which may significantly affect the control data.

In many of the researches in this field the bulk of the control data were obtained from blood banks located in the same city as the hospital residence of the patients. Since the blood bank and hospital, although in the same city, were usually independent organizations often in different areas of the city, the donors were rarely relatives of the patients. As the metropolitan areas which have been the sites of the various investigations are ethnologically heterogeneous, it is unlikely that the blood donors and patients would be homogeneous, ethnologically or by blood groups. The use of such blood donor samples as controls, then, is subject to criticism since differences from the blood type frequencies of the patients may be ethnological ones, not indicating a causal relationship between the patients' blood group and their diseases. To avoid this pitfall, it would be necessary to have precise information concerning the racial, national and geographical origins of all persons, donors and patients alike. Appreciation of the possible contribution that racial stratification has made to the observed differences was one factor prompting the researches of Clark *et al* (1956), in which the relatives of patients with duodenal ulcer were used as controls, rather than other patients or blood donors.

The blood donor controls for our investigations have been relatives or friends of the patients. They have come from the same geographic areas and have similar ethnologic origins. The population of the state of Iowa is relatively stable. There are no large metropolitan areas and few transient residents. These facts reduce the chance of heterogeneity between our blood donors and patients.

Although the blood type frequencies of Controls I (Buckwalter *et al* 1956) and Controls II are similar with no statistically significant differences between them, there are reasons for believing the latter are more representative of the population from which the patients came, and are therefore better controls. Among these are: (1) Controls II were obtained from donors from each of the 17 years from which the patient material was obtained. Ethnological and other changes in the population which might affect the blood type frequencies of the patients should be reflected in the blood donor data. (2) No significant differences with respect to age, sex, or season of year when the blood was collected were noted in the data for Controls II. (3) The effect of including professional donors was observed. (4) The eight-fold larger number in the sample enables the finding of significant differences when otherwise such differences might not be discovered.

Because of the differences in the blood type frequencies of Controls II from Controls I, table 1 (differences not statistically significant), the statistical significance of the findings for several diseases is altered. Chi Square values and probability for the O:A and O:A:B:AB comparisons using the original and new controls, are recorded in table 7. For example, notice that the differences between the controls and patients with stomach carcinoma, pernicious anemia and hip fractures appear less significant, while the findings in lung carcinoma and gastric ulcer appear more significant.

TABLE 7. RESULTS OF THE STATISTICAL EVALUATION OF THE PATIENT DATA USING THE ORIGINAL AND NEW CONTROLS

Patients		Blood Types				Patients: Controls I			Patients: Controls II		
		O	A	B	AB		O:A	O:A:B:AB	O:A	O:A:B:AB
Peptic Ulcer, 1839	No.	983	679	134	43	χ^2	23.258	37.049	38.133	56.777
	%	53.5	36.9	7.3	2.3	Prob.	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Duodenal Ulcer, 1301	No.	698	472	102	29	χ^2	20.340	29.573	30.864	41.943
	%	53.7	36.3	7.8	2.2	Prob.	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Gastric Ulcer, 469	No.	248	183	26	12	χ^2	4.206	12.905	6.373	19.950
	%	52.9	39.0	5.5	2.6	Prob.	$.02 < P < .05$	$.001 < P < .01$	$.01 < P < .02$	$P < .001$
Gastric Carcinoma, 908	No.	383	416	84	25	χ^2	5.623	7.607	3.844	5.439
	%	42.2	45.8	9.2	2.8	Prob.	$.01 < P < .02$	$.05 < P < .10$	$.02 < P < .05$	$P > .10$
Pernicious Anemia, 158	No.	59	76	14	9	χ^2	4.012	6.722	3.219	4.819
	%	37.3	48.1	8.9	5.7	Prob.	$.02 < P < .05$	$.05 < P < .10$	$.05 < P < .10$	$P > .10$
Breast Carcinoma, 866	No.	370	383	81	32	χ^2	2.861	2.877	1.567	1.085
	%	42.7	44.2	9.4	3.7	Prob.	$.05 < P < .10$	$P > .10$	$P > .10$	$P > .10$
Lung Carcinoma, 395	No.	202	144	33	16	χ^2	4.643	5.259	6.635	7.475
	%	51.1	36.5	8.4	4.0	Prob.	$.02 < P < .05$	$P > .10$	$.01 < P < .02$	$.05 < P < .10$
Rheumatic Disease, 254	No.	100	113	26	15	χ^2	2.461	7.339	1.707	6.255
	%	39.4	44.5	10.2	5.9	Prob.	$P > .10$	$.05 < P < .10$	$P > .10$	$.05 < P < .10$
Hip Fractures, 981	No.	410	441	95	35	χ^2	5.312	5.687	3.539	3.616
	%	41.8	45.0	9.7	3.5	Prob.	$.02 < P < .05$	$P > .10$	$.05 < P < .10$	$P > .10$

The question has been asked, and has not been finally answered, whether blood donors do, in fact, provide the best controls for these investigations. Each of the alternatives which have thus far been brought forward has disadvantages which make it less attractive than blood donors. Other patients in the hospital population receiving transfusions, usually have serious disorders themselves which may have an association with blood type. Evidence of associations of varying statistical significance has been found in patients with gastric carcinoma, Aird *et al* (1953), Hollander (1953), Køster *et al* (1955), Jennings *et al* (1956), Billington (1956), Buckwalter *et al* (1957); peptic ulceration, Aird *et al* (1954), Clarke *et al* (1955), Brown *et al* (1956), Clarke *et al* (1956), Billington (1956), Buckwalter *et al* (1956); pernicious anemia, Creger and Sortor (1956), Collective series (Great Britain 1956), Buckwalter *et al* (1956); diabetes mellitus, McConnell *et al* (1956); rheumatic fever, Buckwalter (1957); hip fractures, Buckwalter *et al* (1956); and, pituitary adenomas, Mayr *et al* (1956). It is probable that investigations now in progress will disclose associations for additional diseases. Unaffected siblings, parents, and children of patients provide controls which eliminate population stratification as a possible explanation for the blood group disease associations. However, the accumulation of sufficient data may be impossible and is always difficult. It is unlikely that samples of the population such as hospital visitors, voters, tax payers, or a sample obtained by door to door canvass, would be superior to that provided by blood donors. These sources might provide a better answer to the question concerning the relationship of age to blood type frequencies.

All of these controls are subject to a common criticism tending to invalidate their use. The so-called "healthy" population is so only in the sense that all persons included are free of disease at a given time. However, from this time on, increasingly larger numbers of this so-called "healthy" population develop disease processes, including those diseases being studied. If there is a causal relationship between diseases and blood types, not as yet to be accepted as fact, the disease predisposition exists undetected in "healthy" controls. If this concept is correct, supported by the evidence of associations between the blood groups and a variety of disorders, blood type frequencies of any "healthy" population sample reflect mean frequencies determined by multiple selective factors related to disease and blood types. Therefore, there would be no "healthy" population in this sense, not predisposed to diseases related to blood types, to use as controls.

The patients themselves, may provide the best controls. Observe the striking differences in the blood type frequencies, gastric carcinoma and peptic ulcer, or lung carcinoma and pernicious anemia, table 7. By contrast, note the lesser differences from the blood donor controls. This would be expected, if blood type frequencies observed in blood donors or other heterogeneous samples of the population such as patients with other diagnoses, are means resulting from selection related to diseases and the blood groups. Direct comparison of homogeneous patient groups reveals differences not apparent when patient and blood donor groups are compared.

To establish that differences in blood type frequency unrelated to ethnological factors do exist in patients with various disorders, large numbers of cases must be collected. For example, 395 patients with lung carcinoma showing an increase in

blood type O (table 7), suggests only a need for the collection of more data. It is of the first importance for these patient groups to be homogeneous. Only patients with unequivocal diagnoses should be included.

SUMMARY

The controls are of vital importance to investigations concerned with the association of the ABO blood groups to disease. Data obtained from 49,979 voluntary blood donors have been reported. Use of these data as controls has been discussed. The authors suggest the findings warrant the following tentative conclusions: (1) Large (49,979) numbers of blood donors provided better controls than smaller numbers (6,313). (2) Controls more representative of the population from which the patients came were obtained when the blood donors came from the same time interval as the patients. (3) There was no evidence of a change in the blood type frequencies related to age (18 to 60 years). (4) The blood type frequencies of men and women were similar over-all and in the different age groups. (5) Blood type frequencies did not differ significantly when winter and summer donors were compared. (6) Inclusion of professional donors significantly increased the frequency of blood type O. (7) Homogeneous patient groups may well be better controls than blood donors.

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The Distribution of Blood Group Alleles Among Indians of Southwest North America[1]

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THE EXISTENCE OF INDEPENDENT largely genetic characters such as the human blood agglutinogens has allowed human geneticists and physical anthropologists to learn a good deal about the biological relationships and history of human populations. Extensive studies of the blood groups of modern Europeans have demonstrated the biological influence of Viking raiders two thousand years ago (Mourant, 1954). Evidence of historical migrations and the genetic relationships of population subgroups has been found in the distribution of these traits in the various tribes of Australian aborigines (Birdsell, 1950), and among the inhabitants of Puerto Rico (Thieme, 1952). Intensive sampling in a suspected sociological isolate has demonstrated that the isolation of small groups even in areas of dense population may be a biological as well as a social reality (Dunn and Dunn, 1957). These properties have also been used to examine the internal structure and historical relations of parts of the Apache tribe (Kraus and White, 1956).

In this paper we propose to examine the genetic structure and relationships of several tribes in Southwestern North America using ABO, MN, and Rh data. These tribes provide particularly suitable examples of several properties of population evolution because of their relatively small size and geographic isolation.

METHODS AND MATERIALS

The new blood group data presented in this paper were obtained by sampling the children of the United States Indian Service Schools at Tuba City on the Western Navaho Reservation, Oraibi on the Hopi Reservation, San Carlos on the San Carlos Apache Reservation; Pima and Maricopa children on the Gila River and Salt River Indian Reservations, Mohave and Chemehuevi children on the Colorado River Indian Reservation near Parker, all in the state of Arizona, and the Indian children attending the San Pasqual Union School, Imperial County, California, and the Yuma Union High School, Yuma, Arizona.

In each Indian Service school all of the children were tested. No attempt has been made to classify the individual children of a particular sample according to tribe or degree of racial admixture. The frequencies reported are those of the resident popu-

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lations and not of any select group in the school. Two exceptions have been made to this rule. Because of the large amount of pedigree information about the Salt and Gila River populations which is available, it has been possible to separate the residents of these regions into two tribes, Pima and Maricopa. The data of the group classified as Maricopa contain information on 38 Maricopa Indians (about 9 per cent of the known Maricopa population), and the data of the group called Pima contain information about the Indians on the two reservations who claim to be Pima. From the two public school samples, data are presented only on those children recorded on the school records as Indians.

Each child was interviewed at the time the sample was taken and his name, age, names of parents, sibs, other relatives, and his own statement of his tribal affiliation were obtained. Adult Indian employees of the schools were very cooperative in several instances in supplying tribal and family information.

A school sample is unavoidably biased to include an excess of siblings since children are often closely spaced in time and therefore attending school together. The same is true with respect to cousins since their parents are often similar in age and therefore in childbearing period. If, however, there is no selection by family correlated with blood group genotype, there is no reason to expect that an unselected sample containing an excess of sibs and cousins would be a genetically biased sample of the population as a whole.

Our sample is, in essence, just a chronological slice containing nearly all the children in the age range 6 to 14 of the Navaho, Pima, Maricopa, and Mohave-Chemehuevi populations and the 6 to 16 year age group of the Hopi-Tewa, Apache, and Yuma populations. If the population is stable it should be similar to any other chronological sample. Therefore it is not anticipated that the differences in the mean age of the sample in the different schools will affect the comparisons between them. Other biases may occur in this sample; these are found in any but the most carefully controlled and extensive samples. The possible consequences of these nonrandom deviations will be considered later.

Blood was collected by venepuncture from children over nine years old and by finger puncture from those younger. At least one cc was collected from each child. The ABO tests were run by slide technique at the time of collection; the remainder of the blood tests were done during the evening of the day of collection, either in a temporary laboratory established in the school clinic or in a laboratory at Mesa, Arizona.

Twelve antisera were available for this study project: anti-A, A_2, B, M, N, S, rh′, Rh°, rh″, hr′, hr″, K, and Fy^a. The first three of these were the slide type and the rest were test tube agglutinins. Rh° and hr′ were determined using plasma cell suspensions and the others using saline cell suspensions. The last three antisera listed were available in limited quantities, therefore the anti hr″ was used only to demonstrate the presence of R^z in a population while the anti-K and anti-Fy^a sera were used only on the Pima sample. Anti-S was used only on the Navaho, Hopi, Pima and Maricopa samples.

All tests were read both macroscopically and microscopically. Positive reactions for all sera except anti-B, -rh″, and -K were obtainable by testing the known blood types of two of the investigators. Periodic checks of sera and technique were made

using these bloods. The anti-K serum gave positive reactions with known Kell positive bloods before and after use in the field. A further check on technique and sera was possible by the use of repeat testing with ABO, MN, and Rh sera of Pima and Maricopa individuals previously tested by another investigator (Dr. I. Davidsohn, reported in Hanna *et al*, 1953). These results were in agreement. In addition, ABO, and Rh° tests were made on a sample of 125 white students in an Arizona public school. The frequencies obtained did not deviate from those expected. The anti-hr″ serum used in this study was obtained from the Boston Blood Grouping Laboratory; all other sera were obtained from the Certified Blood Donor Service, Jamaica, N. Y.

RESULTS

The distributions of the ABO blood groups and MNS and Rh blood types in the various populations are given in table 1. The MNS data include only one member of each family studied in the Pima-Maricopa population and only unrelated mem-

TABLE 1. BLOOD GROUP PHENOTYPE DISTRIBUTION IN POPULATIONS OF ARIZONA INDIANS

Phenotype				Tribes				
				West. Navaho	Apache San Carlos	Pima	Yuma	Mohave and Chemehuevi	Hopi and Tewa	Maricopa
O				61	105	395	167	109	115	38
A_1				45	74	93	14	7	3	0
A_2				0	0	0	0	0	5	0
B				0	0	0	1	1	0	0
A_1B				0	0	1	0	0	0	0
Number tested				106	179	489	182	117	123	38
M				30	97	229	97	62	33	10
MN				67	59	220	64	44	62	24
N				7	20	36	19	10	23	4
Number tested				104	176	485	180	116	118	38
MS				0	—	22	—	—	1	0
Ms				4	—	56	—	—	9	4
MNS				4	—	27	—	—	5	4
MNs				16	—	59	—	—	25	9
NS				0	—	2	—	—	2	0
Ns				1	—	6	—	—	10	2
Number tested				25	—	172	—	—	52	19
rh′	hr′	Rh°	rh″							
+	−	+	−	24	47	136	63	—	20	12
+	+	+	−	14	22	59	32	—	23	3
−	+	+	+	29	64	104	34	—	35	10
+	−	+	+	1	2	20	4	—	0	0
+	+	+	+	15	40	139	39	—	7	8
−	+	+	−	4	3	6	7	—	21	0
+		+	+	15	0	22	0	—	6	5
+		+	−	2	0	3	0	—	5	0
Number tested				104	178	489	179	—	117	38

TABLE 2. KELL AND DUFFY PHENOTYPES AND GENOTYPES OF THE PIMA INDIANS

Sample size	Anti-K positive	Anti-K negative	Anti-Fy^a positive	Anti-Fy^a negative	allelic frequency Fy^a	allelic frequency Fy^b	σ
188	0	188	—	—			
184	—	—	5	179	.013	.987	.009

bers of the Hopi-Tewa sample who claimed, or whose names indicated, no Tewa admixture. The MN phenotypes of the individuals tested for MNS are included in the appropriate MN samples reported. The Kell and Duffy phenotypes and the Duffy allelic frequency of the Pima sample are presented in table 2.

These data have been treated by appropriate methods for the estimation of the allelic frequencies of the various tribes. The maximum likelihood method of Stevens (1938) and the Bernstein correction with maximum likelihood estimate of variance following the procedure of Neel and Schull (1954) were used for the ABO data. Simple counting and estimation of variance following Neel and Schull were employed for MN. The MNS and Rh estimates were made by the appropriate methods of Boyd (1954a, 1954b). However in several cases it was possible to make simplifying assumptions about the absence of some of the possible alleles from the population in question so that simpler methods (Stevens 1938, Neel and Schull 1954) were applicable. The estimated allelic frequencies and their standard deviations are given in table 3. Since all of these methods assume a Hardy-Weinberg equilibrium in the sample as well as in the population it is desirable to determine whether this condition prevails in the populations sampled. The expected numbers of the various phenotypes were compared with the observed numbers by a Chi square test following the method of Neel and Schull. The results are presented in table 4.

In just under half of these comparisons the observed and expected phenotype distributions differ significantly. Although part of this difference may be due to poor fit of the estimates, the large bulk of it must be due to non-randomness of the sample and non-random mating in the populations. Kraus and White (1956) have examined the mating structure of the Apache Tribes on the White River Reservation and found that marriage records show random mating within tribal groups as far back as 1800. If this conclusion is applicable to the tribes of this study then it is the structure of the sample which is causing the deviation from expectation.

Another possible explanation for the deviation from expectation is the presence of false positive readings of the serological phenotype. This would tend to increase apparent heterozygosis and produce the type of deviation from randomness observed in the MN data. This explanation is made unlikely for at least part of the data by the excellent reproducibility of the phenotypes of individuals tested in two different laboratories and several years apart in time as was noted in the Pima sample.

The fit of the Rh data in the Apache, Mohave-Chemehuevi and Yuman samples indicates serious deviation from expectation. This may be due to heterogeneity of the sample, but it is possibile that there has been a technical error, particularly in the typing of the Mohave-Chemehuevi sample. These three populations were the last samples in the field project and some of the anti-serum may have become defec-

TABLE 3. THE FREQUENCY OF BLOOD GROUP ALLELES OF POPULATIONS OF ARIZONA INDIANS

Allele	Tribes						
	West. Navaho	Apache San Carlos	Pima	Yuma	Mohave and Chemehuevi	Hopi and Tewa	Maricopa
I^{A_1}	.2414 ±.0316	.2341 ±.0240	.1001 ±.0099	.0392 ±.0103	.0304 ±.0035	.0197 ±.0213	0
I^{A_2}	0	0	0	0	0	.0199 ±.0248	0
I^B	0	0	.0010 ±.0010	.0027 ±.0027	.0043 ±.0013	0	0
i	.7586 ±.0316	.7659 ±.0240	.8989 ±.0099	.9580 ±.0106	.9653 ±.0037	.9604 ±.0347	1.0000
M	.6106 ±.0338	.7188 ±.0240	.6990 ±.0147	.7170 ±.0237	.7241 ±.0293	.5424 ±.0324	.5789 ±.0566
N	.3894 ±.0338	.2812 ±.0240	.3010 ±.0147	.2830 ±.0237	.2759 ±.0293	.4576 ±.0324	.4211 ±.0566
R^z	.0260 ±.0363	.0051 ±.0080	.0508 ±.0058	.0060 ±.0028	—	0	—
R^1	.3753 ±.0479	.3624 ±.0271	.4439 ±.0159	.5666 ±.0217	—	.3135 ±.0344	—
R^2	.2605 ±.0482	.2637 ±.0243	.2627 ±.0109	.2369 ±.0190	—	.2360 ±.0382	—
R^0	.3382 ±.0472	.3687 ±.0238	.1257 ±.0058	.1905 ±.0178	—	.4505 ±.0404	—
r'	0	0	.0813 ±.0162	0	—	0	—
r''	0	0	.0357 ±.0044	0	—	0	—
MS	.1440	—	.1327	—	—	.0646	.0935
Ms	.4160	—	.5707	—	—	.4162	.4591
NS	.2323	—	.1094	—	—	.0799	.1230
Ns	.2077	—	.1871	—	—	.4393	.3244

TABLE 4. THE PROBABILITY THAT THE OBSERVED DATA FIT THE EXPECTED VALUES BASED ON THE GENE FREQUENCIES ESTIMATED AND THE HARDY-WEINBERG EXPANSION

Tribe	Locus			
	ABO	MN	MNS	Rh
West Navaho	>.5	<.005	.005–.001	.5–.4
Apache San Carlos	>.5	.05–.025	—	<.0001
Hopi and Tewa	>.5	>.5	.10–.05	.20–.10
Maricopa	>.5	.10–.05	—	—
Mohave and Chemehuevi	>.5	.60–.50	—	—
Pima	>.5	.10–.05	<.0005	.20–.10
Yuma	>.5	.10–.05	—	<.0005

tive although extensive precautions were taken to prevent this. For this reason the Mohave-Chemehuevi Rh data and calculations have been omitted.

DISCUSSION

The allelic frequencies at the ABO locus, as reported in the literature and in this study are given for tribes of Southwest North America in table 5. In this table are recorded the place where the sample was taken and the language of the population. This table shows the general pattern of the American Indian population as a whole; the allele I^B is essentially lacking and the frequency of the allele I^A is less than 25 per cent except in isolated instances.

The data indicate that the general pattern for this group of tribes is correlated with language. The Athapaskan speaking populations have a higher frequency of allele I^A than other tribes of this area. They have, however, slightly lower frequencies than those reported for the Sarcee, an Athapaskan tribe of Western Canada (Chown and Lewis, 1955). The Shoshonean and Yuman speaking populations have the lowest frequency of allele I^A in the area, the Piman and Tanoan speaking populations are intermediate. However, the Shoshonean, Tanoan, Yuman, and Piman populations are much more similar to each other than they are to the Athapaskan populations.

The Shoshonean and Piman language groups are part of the larger Uto-Aztecan stock, and are probably related distantly to Tanoan. The southern Athapaskan-speaking populations moved into the Southwest 700–900 years ago, breaking off from the main body of Athapaskans in southern Canada. The Yuman language group has no close affiliates in the central southwest but has distant linguistic relatives in California and to the South and East in Mexico.

One striking difference between the Hopi and the other samples was noted. In table 1 it may be seen that five Hopi individuals are of type A_2. These individuals are from the second and third Mesas. The gene I^{A_2} appears to be more frequent than I^{A_1} in this population. That it should have been introduced from the outside is possible in view of the fact that we did observe one child who was half Spanish. It is not likely, however, in light of the complete absence of A_2 in all of the other samples in the area including those with known Caucasian and Negro admixture. The very striking difference between the Navaho and Hopi-Tewa frequencies also is evidence for the high degree of biological isolation of this population.

Within the Athapaskan samples there appears to be an increase in the frequency of the allele I^A from East to West. The Western Navaho and Western Apache bands are both significantly higher in frequency of A than their eastern neighbors. The Uto-Aztecan populations show an opposite, but not as marked, trend. The Pueblo (Tiwa, Towa, Tewa, and Keresan) samples have higher A frequencies than the Yuma, Papago, Mohave, and Mexican samples. This may be due to greater contact of the Pueblo tribes with the Athapaskans, as in the case of the Pima tribe, or to a precontact chance similarity. There is considerable evidence that the Western Apache groups are offshoots of early Navaho populations (Kauti, 1955). This separation may have occurred before contact was made with the Indians already in the area.

The collected data on the distribution of the MN alleles in the Southwestern tribes are not nearly as extensive as are the ABO data. These are presented in table 6. From

TABLE 5. ABO ALLELIC FREQUENCIES OF SOUTHWESTERN INDIAN POPULATIONS

Population	Location	Language Group	Ref. No.†	Sample size	Allelic Frequency		
					I^A	I^B	i
Navaho	Tuba City, Ariz.	Athapaskan	p	106	.241	0	.759
	Ft. Defiance and St. Michael, Ariz.		28	457	.145	.001	.845
	Ramah, N. Mexico		8	361	.124	0	.876
	Ramah, N. Mexico		18	97	.133	0	.868
and Apache	New Mexico		1	622	.166	0	.833
Apache	Cibecue, Ariz.		21	141	.154	0	.846
	East Fork, Ariz.		21	248	.153	0	.847
	Cedar Creek, Ariz.		21	311	.221	0	.779
	Mescalero, N. Mex.		14*	110	.283	.020	.697
	San Carlos, Ariz.		p	179	.234	0	.766
Ute	Ft. Duchesne, Utah	Shoshonean	24, 25	242	.010	0	.990
Hopi	Keams Canyon, Ariz.		31	52	.081	0	.921
and Tewa	Oraibi, Ariz.	and Tanoan	p	123	.040	0	.960
Tewa	San Juan, N. Mex.	Tanoan	3	142	.050	0	.950
Tiwa	Taos, N. Mexico		3	203	.101	.026	.870
Towa	Jemez, N. Mexico		3	310	.105	.007	.885
	Jemez, N. Mexico		2	140	.110	.004	.886
Keresan	Cochita, N. Mex.	Keresan	3	353	.061	0	.939
Pima	Gila and Salt River, Ariz.	Piman	15	97	.097	.006	.898
			p	489	.100	.001	.899
Papago	Sells, Ariz.		9	600	.031	0	.969
Maricopa	Gila Crossing, Ariz.	Yuman	p	38	0	0	1.000
Mohave and Chemehuevi	near Parker, Ariz.	and Shoshon.	p	117	.031	.004	.965
Yuma	near Ft. Yuma, Cal.	Yuman	p	182	.039	.003	.958
Diegueno	San Diego co. Cal.	and Shoshon.	29	58	.009	.009	.983
Tolteca	Tuxpan, Mexico	—	35	98	.031	.015	.954
Tarasco	Mexico, D.F. Mexico	—	4	111	.062	.016	.927
Otomi	Mexico, D.F. Mexico	—	4	81	.031	0	.969

* sample contained 8 non Apache Indians, 3 non Indians, and 4 half Indians. Our estimates do not allow for this mixture.

† p = present study

a comparison of the new data with other blood group information about Southwestern Indians we see that they are similar in that the frequency of the *M* allele is uniformly higher than that of the *N*. This is typical of American Indian frequencies in general. Our data differ, however, in being less extreme in their *M* frequencies than most of the other samples from this region.

Table 4 shows that the calculated Western Navaho *MN* allele frequencies give a very poor fit to the data as observed, even though this calculation involves the minimum of estimation error since it is a matter of simple gene counting in the sample. The lack of fit is probably a matter of the structure of the sample which may also account for the borderline significance value found in the San Carlos Apache sample.

Because of the lack of sufficiently numerous or large samples it is not yet possible to make clear correlations between frequencies of *MN* alleles and particular popula-

TABLE 6. THE MN ALLELIC FREQUENCIES OF SOUTHWESTERN INDIAN POPULATIONS

Population	Location	Language Group	Ref. No.†	Sample size	Allelic Frequency		
					M	*N*	Std. Dev.
Navaho	Tuba City, Ariz.	Athapaskan	p	103	.611	.389	.034
	Ramah, N. Mexico		8	361	.917	.083	.010
	Ramah, N. Mexico		18	97	.887	.113	.023
Apache	Mescalero, N. Mex.		14*	110	.827	.173	.025
	San Carlos, Ariz.		p	176	.719	.281	.028
Ute	Ft. Duchesne, Utah	Shoshonean	24	104	.760	.240	.030
Hopi and Tewa	Oraibi, Ariz.	and Tanoan	p	116	.542	.458	.032
Towa	Jemez, N. Mexico	Tanoan	2	140	.757	.243	.026
Pima	Gila and Salt	Piman	15	97	.713	.287	.040
	River, Ariz.		p	485	.699	.301	.015
Maricopa	Gila Crossing, Ariz.	Yuman	p	38	.579	.421	.057
Mohave and Chemehuevi	near Parker, Ariz.	and Shoshon.	p	116	.724	.276	.029
Yuma	near Ft. Yuma, Cal.	Yuman	p	180	.717	.283	.024
Diegueno	San Diego co., Cal.	and Shoshon.	29	58	.756	.244	.040
Tolteca	Tuxpan, Mexico	—	35	98	.791	.201	.029
Tarasco	Mexico, D.F. Mexico	—	4	111	.847	.153	.024
Otomi	Mexico, D.F. Mexico	—	4	81	.864	.134	.026

* sample contained 8 non Apache Indians, 3 non Indians, and 4 half Indians. Our estimates do not allow for this mixture.

† p = present study

tion groups. Within the Athapaskan samples, however, there does seem to be a significant increase in the frequency of *M* in the eastern samples which are close to the Tanoan and Keresan populations. There also appears to be a cline of increasing *M* from north to south along the west coast of Mexico.

The values obtained in this study for the frequency of the alleles of the Rh locus are qualitatively similar in frequency of R^1, R^2, and R^z to the values of the other samples obtained from this area as is shown in table 7. They do differ in the frequencies of the R^o allele. The distribution of Rh alleles in the West Navaho and San Carlos Apache differ from the values obtained by Boyd (1949) at Ramah, New Mexico, and by Kraus and White (1956) on the White River Reservation in Arizona, but are similar to the values obtained by Mourant (1952) at Ramah. The phenotype distributions are similar to the distribution reported by Chown and Lewis (1955) for the Sarcee. The Apache also resemble the Sarcee in A frequency. The frequency of the alleles of the *r* series in these populations is so low that it can be explained by the recent introduction of these genes by European immigrants into the area.

At the Rh locus the Athapaskan populations, as a whole, differ from the Uto-Aztecan group in their frequency of the allele R^1. The Piman, Yuman, and Mexican frequencies are all well over 0.5. The Ute R^1 frequency is just over 0.5, and only in the Hopi-Tewa sample of the Shoshonean populations is it under 0.5. In contrast, all of the Athapaskan samples have R^1 allelic frequencies below 0.5.

The MNS data are of doubtful significance because of their large deviation from

TABLE 7. THE RH ALLELIC FREQUENCIES OF SOUTHWESTERN INDIAN POPULATIONS

Population	Location	Ref. No.†	Sample size	Allelic Frequency					
				R^a	R^1	R^2	R^0	r'	r''
Navaho	Tuba City, Ariz.	p	104	.026	.375	.261	.338	0	0
	Ramah, N. Mexico	8	305	.058	.326	.356	.069	.172	.019
	Ramah, N. Mexico	18	97	.013	.431	.277	.280	0	0
Apache	Cibecue, Ariz.	21	36	.125	.403	.403	.069	0	0
	East Fork, Ariz.	21	231	.145	.467	.346	.028	(.013)	
	Cedar Creek, Ariz.	21	114	.131	.465	.381	.013	(.009)	
	Mescalero, N. Mex.	14*	110	.016	.496	.411	.067	(.010)	
	San Carlos, Ariz.	p	178	.005	.362	.264	.369	0	0
Ute	Ft. Duchesne, Utah	24	104	0	.524	.476	0	0	0
Hopi and Tewa	Oraibi, Ariz.	p	117	0	.314	.236	.450	0	0
Pima	Gila and Salt River	15	97	.046	.583	.358	.013	0	0
	Reservation, Ariz.	p	489	.051	.444	.263	.126	.081	.035
Maricopa	Gila Crossing, Ariz.	p	38	0	.547	.371	.082	0	0
Yuma	near Ft. Yuma, Cal.	p	179	.006	.567	.237	.191	0	0
Diegueno	San Diego co., Cal.	29	58	.059	.683	.234	.024	0	0
Tolteca	Tuxpan, Mexico	35	98	0	.681	.300	.019	0	0

* sample contained 8 non Apache Indians, 3 non Indians, and 4 half Indians. Our estimates do not allow for this mixture.

† p = present study

their expectation in the Pima and West Navaho samples. They are generally different from other Indian samples reported in their relatively high frequency of the allele Ns.

The absence of Kell positive individuals among American Indians, as reported by other workers (Pantin and Kallsen, 1953; Chown and Lewis, 1953, 1955) was confirmed by our results. The frequency of Duffy (Fy^a) observed in the Pima is intermediate between the high value reported for the Diegueño and the absence of Fy^a reported for the Sarcee, but is similar to that found among the Mescalero (Gershowitz, 1957).

A preliminary attempt at representing the genetic structure of the Southwestern Indian populations in the form of 5 per cent isogene maps of the three loci is presented in figure 1. Of these three maps only the map of the $I^A{}_1$ gene is based on a sufficient number of points to be reliable as to contour, but as compared with the linguistic map they serve to point out the general picture as well as the great gaps in information about the blood groups of this area.

These maps show clearly the genetic differences of the Athapaskan speaking populations from their Uto-Aztecan neighbors. Within the Athapaskan populations gene frequencies tend to approach those of nearby populations of different linguistic grouping, particularly towards the east. This gradient within the Athapaskan populations may represent evidence for genetic interaction bewteen these populations and their Pueblo neighbors. There is also indication of a distinction between the Uto-Aztectan tribes of north Mexico and the United States and the long time residents of central and south Mexico who are linguistically distinct. The Tuxpan sample is of particular interest in this respect because this population has a history of migration from the

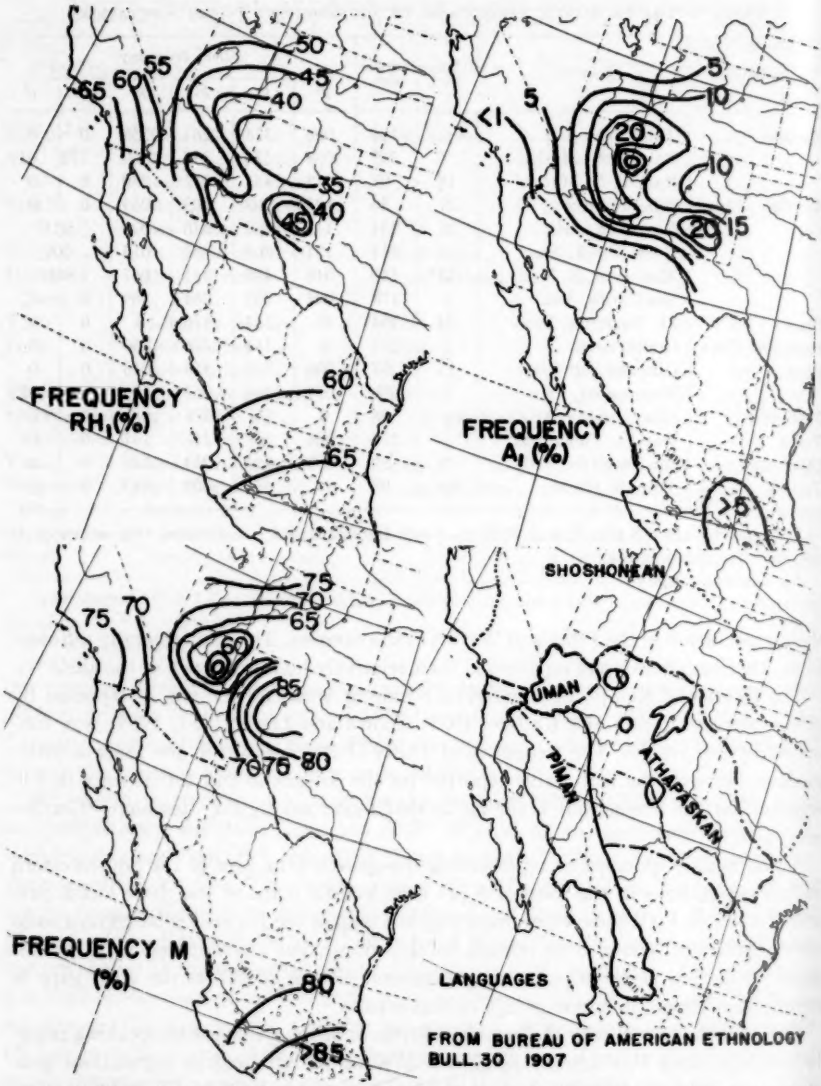

FIG. 1. Maps showing the 5 per cent isogenes of alleles of the ABO, MN, and Rh loci and the distribution of indigenous language groups.

north in the post Christian era, although at present this group lives in the same region as other Mexican Indian populations (Otomi, Totonaco, etc.) they are genetically more like Uto-Aztecan tribes of the southern United States than they are like the long time inhabitants of this region.

The Pima tribe of the Gila and Salt River valleys occupies the extreme northern tip of the Piman language group and, as can be seen from the language map of figure 1, is also at the junction of the Yuman and Athapaskan language groups. This unique position places it in a particularly interesting relation to the surrounding populations in that it is exposed to attack and possible genetic influence from two populations of considerable genetic difference.

This Pima population has been the prime focus of this study because one of us, A. A. Dahlberg, has been engaged in a long time study of dental morphology and development in this tribe with particular interest in the genetic aspects of some of the tooth patterns observed in this population.

The relation of the allelic frequencies of the Pima tribe to the other tribes sampled in the area is shown in figure 2. Here we have added the Diegueño values as an example of a Yuman population which has been little influenced by possible interaction with the Pima. Lessa (1953) has pointed out that the Diegueño are not Piman speaking as reported by Pantin (1953) and that this sample may contain an element of indigenous Shoshonean individuals. However, its geographic location makes it extremely suitable for this comparison. The West Navaho population is included as an Athapaskan speaking group which has had no historical contact with the Pima. San Carlos Apache and Yuma Indians are known historically to have attacked and captured slaves from the Pima tribe.

This graphical representation (Fig. 2) shows that the Pima allelotype is intermediate between the Athapaskan and Yuman types. The Athapaskan and Yuman tribes which are known to have had contact with the Pima are genetically more like the Pima and therefore similar to the populations on the other side of the Pima, in both a genetic and geographic sense, than are the populations which have not had this contact.

The question is therefore raised: has there been gene flow between these tribes? In an attempt to evaluate the likelihood that these tribes have been interacting genetically we may take advantage of the genetic independence of the three loci (ABO, MN, Rh). The traits controlled by these loci should not show correlated changes in populations unless there are common influences such as migration or cross-mating acting upon them. If these populations were genetically independent and drift were occurring, we would not expect to find any correlation between the changes in frequencies at various loci in a progression from tribe to tribe.

Figure 3 is a representation of the allelic frequencies of the populations sampled as taken from an arbitrary base frequency for each locus and plotted against a scale selected to make the ABO data linear. In the case of random association between these tribes there is no reason to expect that either the MN or the Rh data would be made linear by this particular arbitrary scale or that the absolute value of the slopes of the regression lines on these two sets of data would be in any way similar to the slope of the ABO data.

As may be seen from figure 3, the MN and Rh data give a good approximation to linearity on this scale and their slopes are indeed very similar in absolute magnitude to the slope of the ABO data. A comparison of the slopes of the three regression lines shows that they differ significantly ($P = .005$). That they should indeed be identical

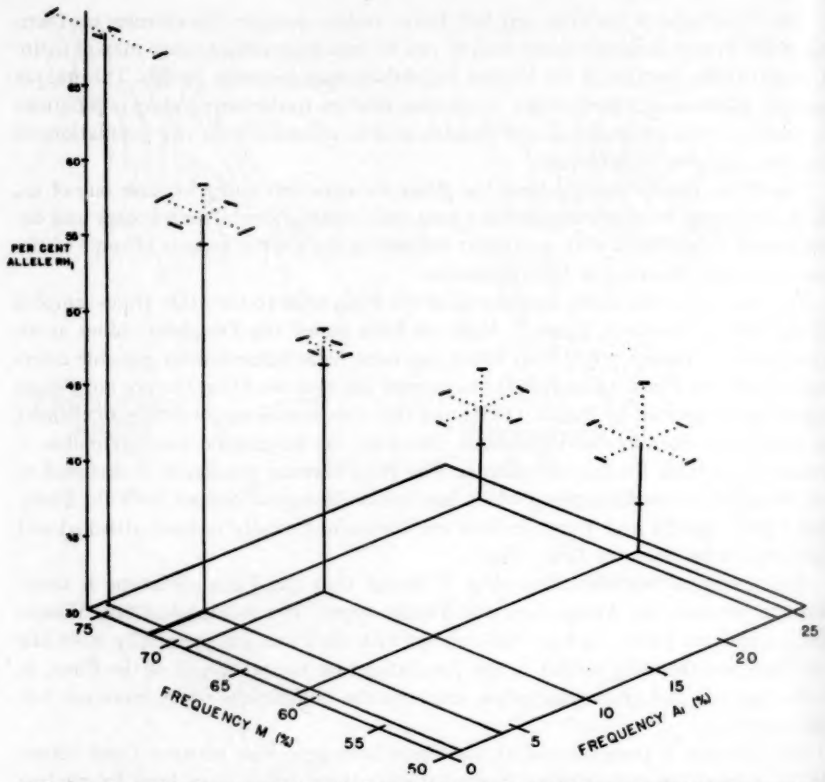

FIG. 2. A graphical representation of the relations of the allelotypes of selected southwestern Indian populations using alleles of the ABO, MN, and Rh loci. The frequency of the gene M of the MN locus is expressed on the axis to the left and the frequency of the gene I^{A_1} of the ABO locus on the axis to the right. The frequency of the gene R^1 of the Rh locus is given on the vertical axis. The standard deviation of each frequency estimate is represented as a dotted line on each side of the estimate in the plane of the appropriate axes. The populations represented are from left to right; Diegueño, Yuma, Pima, San Carlos Apache, and W. Navaho.

would deny the presence of any sampling variation or of drift at these loci within the sampled populations. Their great similarity indicates that some common evolutionary factor has been affecting these tribes. Since this effect is similar in magnitude in all of these tribes gene flow between them seems to be the simplest explanation for their relationships.

If we accept the evidence that there has been gene flow between the Athapaskan and Yuman populations through the Pima tribe, what, then, does the Pima allelotype of today represent? Is it the allelotype of the Hohokam people, the cultural ancestors of the modern Pima and Papago tribes? Is it this ancient allelotype as slightly modified by exogamy? Or is it a new allelotype produced by the mating of surrounding populations with ancestral Pima, this exogamy having been so extensive that the ancestral type has been modified beyond recognition.

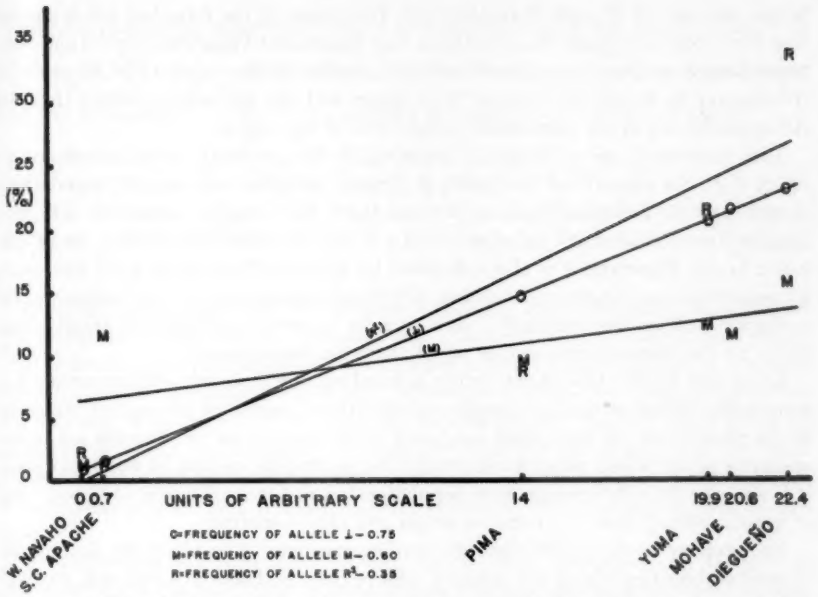

FIG. 3. A graphical representation of the percentage change in allelic frequency of the alleles at independent loci among the W. Navaho, San Carlos Apache, Pima, Mohave-Chemehuevi, and Diegueño. The horizontal scale is arranged so that the change of frequency of the gene *i* of the ABO locus has a slope of unity.

The primary evidence which could be brought to bear on the problem of the ancestral Pima allelotype would be extensive samples from the Papago at all three loci. The Papago is a desert dwelling tribe living on the United States-Mexico border. They presumably have not been subjected to the influence of other tribes to the same degree as have the Pima. Other Piman speaking tribes such as the Opata of the Sonora Valley of Mexico would also provide evidence as to the ancestral Pima allelotype. The only evidence of this type is at the ABO locus in the Papago (table 5). Here we see that the Papago have a significantly lower frequency of allele I^A than do the Pima. Their frequency is very similar to that of the Tolteca sample from Tuxpan, Mexico, and the Yuma and Mohave-Chemehuevi of the Colorado River valley.

For further evidence we must again examine the frequencies at the three loci in the Pima tribe relative to the surrounding populations. This has been done by assuming that the West Navaho and Diegueño are two extremes representing relatively unmodified ancestral allelotypes. If we consider the difference between these extremes as 100 per cent of the possible range of genetic frequencies producible by crossmating, then the relative position of the frequencies at these three loci in the Pima would give an estimate of the degree of relationship this tribe has with each of the two presumed ancestral populations on the basis of migration and intermarriage alone.

The values calculated for the constitution of the Pima on this basis are 61, 65, and

60 per cent for I^A, R^1, and M respectively. The values at the three loci are so similar that they seem to support the hypothesis that the present Pima allelotype is one composed largely of genes introduced from the outside in the rough ratio 40 per cent Athapaskan to 60 per cent Yuman. This agrees with the historical evidence that the Athapaskans are much more recent inhabitants of this region.

This hypothesis gives biological meaning to the arbitrary scale introduced in figure 3. It is a measure of the degree of genetic difference between the populations sampled, or the biological distance between them. It is roughly correlated with geographic distance, as might be expected in a situation where distance is a major isolating factor. However, it is also influenced by ecological barriers to gene flow, such as mountains and deserts, by cultural differences represented by the languages, by ecological and cultural pathways, such as river systems and linguistic affinity, and lastly by the length of time during which there has been contact.

Kraus and White (1956) have rightly pointed out the problems of interpreting in a meaningful biological manner samples taken without regard for the mating structure of the population. As they point out, none of the samples of the Navaho are representative of the whole tribe. As shown by the geographic picture of the allelotypes, it is not a genetically homogeneous population but rather a collection of populations of genetic affinity due to a common origin and cross-breeding.

The problem of the validity of gene frequency estimates based on the assumption of random breeding within the sampled area or tribe is therefore a real one, particularly in evaluating the data in the literature and that procured by school sampling of the type carried out in this study. If several random breeding isolates have been sampled, and the samples unknowingly combined, will the normal estimating procedures give an estimate of the weighted mean of these samples and therefore an estimate of the mean regional allelic frequency or will a biased estimate result?

Consider the simplest case, two alleles with no dominance. The random sample of size n from a random breeding population with frequency p for gene A and frequency q for gene a, where $p + q = 1$, would be expected to be constituted as follows:

$$np^2(AA) + 2npq(Aa) + nq^2(aa)$$

We estimate the frequency of genes A and a by p' and q', where r, s, and t are the observed numbers of the three classes AA, Aa and aa respectively, by $p' = \frac{2r + s}{2n}$ and $q' = \frac{2t + s}{2n}$. The expected numbers are, $E[r] = np^2$, $E[s] = 2npq$, and $E[t] = nq^2$ so $E[p'] = p$ and $E[q'] = q$.

Suppose the sample is composed of two random samples of size n_1 and n_2 where $n_1 = n_2$ and $n_1 + n_2 = n$. Suppose n_1 is taken from a population with gene frequency $(p + x)$ for A and $(q - x)$ for a and n_2 from a population with frequency $(p - x)$ for A and $(q + x)$ for a. Let $r = r_1 + r_2$, $s = s_1 + s_2$, and $t = t_1 + t_2$. If these two populations are individually distributed in a random manner, $E[r_1] = n_1(p + x)^2$, $E[r_2] = n_2(p - x)^2$, $E[s_1] = 2n_1(p + x)(q - x)$, $E[s_2] = 2n_2(p - x)(q + x)$, $E[t_1] = n_1(q - x)^2$, and $E[t_2] = n_2(q + x)^2$. If we estimate p by p' we find that $E[p'] = p$ so no bias is introduced.

Suppose that in the same conditions A is dominant over a. We then estimate p as p'' where $p'' = 1 - \sqrt{\frac{t}{n}}$ and estimate q as q'' where $q'' = \sqrt{\frac{t}{n}}$. For a single sample $E[q''] = q$ since $E[t] = nq^2$. If two samples n_1 and n_2 are combined as above

$$q'' = \sqrt{\frac{t_1 + t_2}{n_1 + n_2}} = \sqrt{\frac{t_1 + t_2}{n}} \quad \text{but}$$

$$E[q''] = \sqrt{\frac{n_1(q - x)^2 + n_2(q + x)^2}{n}} = \sqrt{\frac{(q - x)^2 + (q + x)^2}{2}} = \sqrt{q^2 + x^2}$$

The above shows that when two samples of differing allelic frequency are combined and estimated as one sample, the result is a biased estimate if there is dominance. This is because the proportion of homozygotes is not a linear function of the gene frequency. Thus, a combination of two populations can lead to a significant error[4]. For example: if the frequency of a recessive allele in two truly random mating isolates differs by 0.2, with one frequency 0.9 and the other 0.7, the estimate of the mean allelic frequency, based on equal samples, will be 0.025 greater than the true mean. Thus an estimate of 0.825 instead of 0.800 will result from a sampling error of this type.

A possible way of estimating this condition in the no dominance case is found in the reduction of the number of heterozygotes to be expected in a combined sample. The number of heterozygotes to be expected is reduced by the square of one half the difference in the true population frequencies. This reduction in heterozygotes is independent of the gene frequencies themselves. Thus, if one of the alleles is rather rare, this reduction can be quite apparent. It may possibly be confused with the reduction in heterozygosity to be expected if there is a high coefficient of inbreeding.

Examination of the proportion of heterozygotes in the MN data recorded in the literature does not show any significant deviations of this type. We may therefore safely use these data as representative of the geographic location from which they were taken, if not as representative of the whole tribe from which the sample was taken.

In sampling school children extreme heterogeneity is not so likely to occur as in

[4] Dr. Herman Slatis has pointed out that, in the simple dominance case, the general form of the relation between the value estimated from combined samples (q'') and the true value (q) is

$$q'' - q = \frac{n_1 n_2 (q_1 - q_2)^2}{(n_1 + n_2)^2 (q'' + q)}$$

He reports that this expression has the following properties: (1) q'' is always greater than q, (2) The difference between q'' and q is greatest if $n_1 = n_2$, and (3) The difference is proportional to $(q_1 - q_2)^2$. Dr. Newton E. Morton has reduced this expression to its most general form.

$$q'' - q = \frac{\sigma_q^2}{(q'' + q)}$$

where $\sigma^2 q$ is the n-weighted variance of the gene frequency among populations. This holds for any number of populations in arbitrary frequencies.

the sampling of adults since the school population is the product of the stable part of the adult population and is not so likely to include migrant individuals. However, where boarding schools are sampled as in the case of the West Navaho, the problem of the origin and heterogeneity of background of the individuals again arises. By considering the geographic origin of individuals in the sample some evaluation of any violent local differences may be made.

The West Navaho sample is definitely heterogeneous in origin. Two or three individuals from each of twenty or more small semi-isolates scattered over the west end end of the Navaho reservation from north to south contribute to the total. These small subgroups have been collected into four larger geographical collections representing the north, north-west, south, and east parts of the Western Navaho Reservation. These larger subgroups were analyzed to detect excessive heterogeneity but the sample size was not large enough to allow the determination of any internal or regional variation. It was found that the largest excess of the MN phenotype occurred in the smallest sample of these groups while the smallest excess was found in the largest group. This would indicate that this excess is due to accidental sampling fluctuation although the expectation for such a sampling procedure is that it will reduce the observed number of heterozygotes rather than increase it as has been observed.

Among the Hopi a problem of this type arises because of the high degree of isolation of the various villages on the tops of the individual Mesas even though the villages are much less isolated by distance than the Navaho semi-isolates. Of the four Hopi sub-populations, the three Mesas and the villages of Hotavilla, compared, one showed a low frequency of heterozygotes and one high. These individuals were sampled at random in the school population and therefore a systematic error such as defective typing sera could not produce the results observed.

Hotavilla which showed the greatest deficit of MN phenotype was the smallest sample (18) while the next smallest sample, first Mesa (23), showed the greatest excess of heterozygotes.

The Hotavilla population split off from Oraibi in 1906. It has not had much contact with the Navaho. First Mesa, on the other hand, has considerable Navaho intermarriage, some Zuni and a large amount of Tewa mixture from the refugee groups which settled at Hano around 1700 A.D. The Tewa undoubtedly had some intermixture with Keresan and probably with plains Indians and Spaniards in the Rio Grande region before 1700. Since these frequency deviations are in the direction opposite direction to that expected if due to sampling of several populaitons, this would tend to indicate that these deviations were due to small sample variation, exogamy, or other effects causing a deviation from the Hardy-Weinberg equilibrium. The total Hopi sample did not deviate from expectation significantly.

The total Hopi sample was also examined to detect any significant difference between the Hopi proper and the Tewa or Hopi-Tewa groups. Eleven individuals, all from Polacca, reported that they were Tewa or part Tewa but only one said both parents were Tewa. This small group did not differ from the Hopi proper. It may be noted that three children reported some Navaho or Apache ancestry and one claimed to be part Toas. This may indicate increased mobility with a breakdown of

tribal barriers among these tribes. Although there has been a limited amount of Navaho intermarriage on First Mesa, this has been largely confined to Navaho males marrying Hopi women. These marriages are often shortlived because the cultural division of labor is so different that women of either group cannot easily adjust to the other.

The San Carlos Apache sample is at the border of significance in the degree of fit at the MN locus (table 4) due to a reduced frequency of heterozygotes. This may be due to the sampling of more than one isolate, as discussed above, or to inbreeding in the population, or both. The evidence, on a historical ground, for the presence of more than one tribal band on this reservation is very good. If Kraus and White are correct that the band is an effective isolate then it seems likely that this reduction is due to sampling error. If it is entirely due to sampling two different populations then the MN data indicate that the true isolate frequencies differ between 0.1 and 0.2 from each other. This is possible in light of the band differences observed by Kraus and White at the White River Apache Reservation directly to the north, which were of the order of 0.07 at the ABO locus.

The differences between the San Carlos Apache sample and the West Navaho, and the San Carlos Apache and Ramah Navaho are both between 0.1 and 0.2 at the MN locus. Such differences are therefore possible at this locus also, although the between band differences might not be as large as the between tribe differences. On the other hand, if the bands are as effective isolates as Kraus and White believe, there is no reason why the between band differences should be of a different order of magnitude than the between tribe differences already observed.

A comparison of the Hopi and Pima in regard to their relations with surrounding populations shows an interesting difference. Whereas the Pima tribal allelotype has been strongly influenced by the allelotypes of surrounding tribes, the Hopi allelotype is strikingly different from the surrounding Athapaskan allelotypes. On the basis of simple observation these differences appear to be correlated with the degree of social cohesiveness and tribal identity of these two populations. Both have been exposed to contact with populations very different in allelotype from their own, but the population which is known to possess a high degree of ingroup feeling and sense of community importance has resisted the possible genetic as well as cultural influences of surrounding tribes.

The picture of the Hopi in relation to the Navaho is very similar in its genetic and sociological implications to that presented by Dunn and Dunn (1956) in their description of the Jewish community of Rome. The Hopi have some trade with the Navahos, but social contact is extremely limited and the feeling of difference among the Hopi extends very strongly down into the children as we observed during our stay at Oraibi.

The Pima are different from the Hopi in their social reactions to outside populations. They are more flexible in their social responses to the environment. They have adopted many of the ways of the European residents of the area. They intermarry with Europeans and with Indians of other tribes, at least in the part of their population where social and economic contacts have been high (Hanna, Dahlberg, Brown, and Strandskov, unpublished), even though they show evidence of inbreeding in the

TABLE 8. THE ALLELOTYPE RANGES FOR LINGUISTIC GROUPS IN SOUTHWESTERN NORTH AMERICA (MEASURED IN PER CENT)

Language Group	1^{A_1}	M	R^1
Shoshonean	0–5	55–75	35–55
Yuman	0–5	60–75	55–65
Piman	5–10	70–80	50–60
Athapaskan	15–30	60–70	30–50
Tanoan	0–5	75–95	?
Mexican Indians	0–10	75–85	>60

tribe as a whole. If this relative adaptability, as compared with the Hopi, to external influences is a cultural trait which has persisted from Hohokam times it may help to explain the apparent genetic influences of outside populations on the Pima allelotype.

The effect of genetic drift has not been found to be a necessary major influence in producing the present allelic picture in the southwestern American Indians. It appears that two or possibly three basic allelotypes were originally present or migrated into the area and that the present tribal distributions can be explained in terms of gene flow between these basic populations.

That language can be used successfully as an indicator of biological affinity is demonstrated in table 8. Here it may be seen that each of the linguistic groups, each including several tribes, is represented by a distinctive allelotype range. If a population is sampled in this area it is possible to make a prediction of the langugae spoken by the individuals composing it on the basis of their collective allelotype and conversely. Of course, there are borderline cases, as in any intraspecies distribution, but the general picture is now beginning to take form.

Additional strong support for the view that linguistic groups represent biological units is found in the study of Hanna and Dechert (1957) on quantitative anthropometric measurements, each presumably affected by the interaction of several gene systems. They found, in an analysis of seven Athapaskan, Piman and Yuman tribes, that the linguistic groups were distinct and that each group shows a different degree of internal variation.

Table 8 also has a bearing on the linguistic controversy between Powell (1891) and Kroeber (1907) about the reality of the Uto-Aztecan linguistic grouping. Kroeber contended that these tribes constitute branches of a single stock, linguistically, whereas Powell considered that the Shoshonean family was a similar but independent type. On the basis of the blood group picture Kroeber's general position appears to be biologically correct. The Uto-Aztecan tribes seem related in their blood group allelotypes but this relation may be an ancient one since there has been considerable drift, particularly at the MN and Rh loci. The relative position of the Shoshonean allelotype does indicate that it is more distinct from the others than they are from each other and this supports Powell's position. Glottochronological studies estimate the time of separation of the major Uto-Aztecan languages at about 3500–4000 years ago (Swadesh et al, 1954).

The type of semi-isolate as apparently found in the Navaho, in which several random breeding populations are incompletely isolated from each other by distance,

is the type of population structure which Wright (1943) has concluded leads to high evolutionary rates. As population size increases these drift changes are succeeded by interactions between isolates due to increasing contacts of the different divergent stocks produced by the original drift mechanism.

SUMMARY

Data are presented of the frequencies of the alleles of the ABO, Rh, and MN loci for samples of school children from the West Navaho, San Carlos Apache, Hopi-Tewa, Pima, Maricopa, Yuma, and Mohave-Chemehuevi populations in Arizona and California. Additional data for the Kell and Duffy loci, and the S agglutinin of the MNS system are presented for the Pima tribe. S data are also presented for the West Navaho, Hopi-Tewa, and Maricopa tribes.

Isogene maps have been prepared from these and collected data from the literature for these three loci. The implications of the maps for the ideas about evolutionary changes among the tribes and smaller isolates of the southwest part of North America are discussed. Linguistic patterns are correlated with the allelic picture.

The genetic relations of the Pima tribe to the surrounding tribes are examined and the conclusion is drawn that the present Pima allelotype is a product of an extensive gene flow between this tribe and its neighbors. This influence has been detected in correlated changes in all three loci in the Pima, Yuma, and Apache tribes sampled.

Problems of sampling in this type of a study, with a preliminary evaluation of the degree of bias which might be introduced by combining samples of two isolates and evaluating them as one, are included. A bias is produced in a simple two allele system only in the presence of dominance and is then a function of both, the true gene frequency and the square of the difference between the true frequencies of the populations actually sampled.

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Congenital Deafness Due to a Sex-linked Recessive Gene

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A SIXTEEN YEAR OLD deaf lad (D-32, Fig. 1), involved in petty crime, was referred for psychiatric opinion. During the interview with his parents, it was revealed that two cousins, also born deaf, had been committed to state institutions following anti-social conduct. A detailed study of the family was initiated, revealing that fourteen male members have been deaf from birth, and there is sufficient evidence to establish that this is due to a sex-linked recessive gene.

It is well known that more than one third of the cases of congenital deafness are due to hereditary factors, and investigations into this aspect have been the subject of wide interest since. A. G. Bell, inventor of the telephone, first wrote on this topic in 1884. When van Egmond reviewed the literature in 1954 he found that two varieties of hereditary congenital deafness had been described: the commoner, due to a recessive gene, often made manifest following consanguineous marriages, and a rare variety due to a dominant gene which also affects other characters. Waardenburg (1951) who described the latter has gathered only sixteen pedigrees of this anomaly.

Sataloff *et al.* (1955) outlined a family group which suggested that the seven male cases of deaf-mutism were transmitted in a manner similar to that seen in haemophilia, and their study of the literature failed to disclose any previously reported cases of sex-linked congenital nerve deafness. It is possible that their family and the one now described stem from a common ancestor, as the audiogram patterns closely approximate in both groups; this is being investigated at present.

GENETIC ASPECTS

In June 1888, three sisters (figure 1 A) migrated from Aberdeen and settled in Queensland. They came from a large family, descendants of whom are being traced by Dr. John McKenzie, Department of Anatomy, University of Aberdeen. All three married, the eldest having five daughters and a deaf-mute son (B-5, figure 1). Three of these daughters had deaf sons and four grand-daughters have given birth to deaf sons. Only one of the affected males married, and his wife (who has a recessive form of hereditary deafness) has borne him a son with normal hearing (D-23, figure 1).

There is only one apparent inconsistency, in that a female was born deaf (D-17, figure 1). There is, however, sufficient evidence to suggest that she is a result of the union between a deaf male and his sister. She was born three months after her mother's marriage, has the same severe bilateral perceptive deafness most marked at 1,000 cycles and 4,000 cycles per second characteristic of the group. Her mother was the only member of the family whose co-operation was difficult to obtain, and

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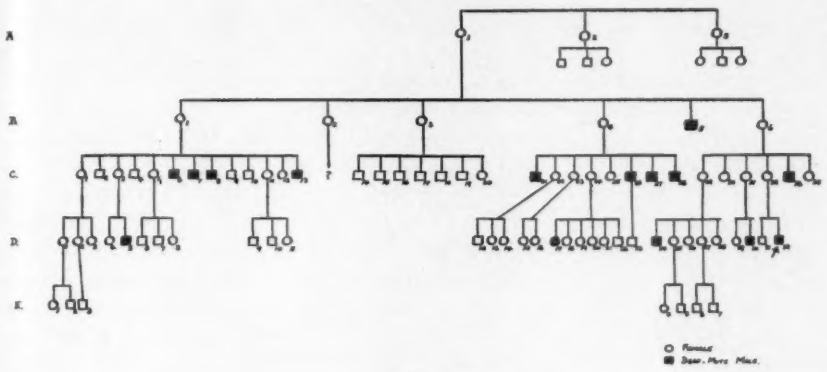


FIG. 1

she had given various excuses in different places to explain her daughter's deafness. One such excuse—that she contracted rubella in the second month of pregnancy—had to be seriously considered. The child was born in 1938, the year following the severe Queensland epidemic, but it was noted that she arrived six months later than most of the affected babies; her mother must have contracted the infection well out of season. The girl shows none of the other stigmata of rubella, so the weight of evidence favours the possibility that she is a homozygous carrier of the gene.

An analysis of the pedigree, following the lines adopted by Aldrich, Steinberg, and Campbell (1954) is summarized in table 1 and, considering the small numbers involved, shows a reasonable approximation between the predicted and observed values.

The expected numbers were calculated as follows: It is obvious that the mother

TABLE 1. EXPECTED AND OBSERVED NUMBERS OF AFFECTED AND NORMAL SONS OF A.1 AND OF EACH FEMALE RELATED TO HER VIA FEMALES

	Sons			
	Affected		Normal	
Female*	Expected	Observed	Expected	Observed
A.1	0.5	1	0.5	0
B.6	0.5	1	0.5	0
B.1,3,4	4.5	8	13.5	10
C.29,31	0.5	2	1.5	0
C.3,5,11,22,24	0.875	1	6.125	6
D.25,27	0.375	0	2.625	3
D.1,2	0.125	0	1.875	2
	7.375	13	26.625	21
A.2,3†	0.75	0	2.25	3
	8.125	13	28.875	24

* Symbols refer to Figure 1.

† If mother of A.1 was heterozygous.

(C-32), grandmother (B-6), and great-grandmother (A-1), of the proband (D-32), must have carried the gene for it to be transmitted to him.

The probability that A-1 would pass it on to any of her daughters, (other than B-6), is one half. The probability that these four women (B-1, 2, 3 or 4), would transmit the gene if she has it, to her sons is again half for each son. The overall probability therefore that a grandson of A-1 would inherit the gene from her, via B-1, 2, 3, or 4, is one quarter, hence one quarter of these grandsons would be expected to be affected.

Likewise the probability that a great-grandson of A-1 born to females C-3, 5, 11, 22, or 24 would inherit the gene is one eighth and in the next generation, one sixteenth.

Since B-6 is known to carry the gene, (because of the direct blood relationship to the index patient), the probabilities of her transmitting the gene is double those of her sisters B-1, 2, 3 or 4 discussed above.

On these calculations seven affected and 27 normal sons could be expected which approximates to the observed number of 13 affected males (excluding the proband) and 21 normals.

Audiograms of their mothers showed no tendency suggestive of partial expression of the gene, and extensive study failed to reveal any feature of value in predicting the carrier females.

METHOD OF STUDY

One deaf-mute (C-7, figure 1) died in infancy, another (C-8, figure 1) due to low intelligence was unable to cooperate; the remainder have been tested audiometrically. In addition twelve were subjected to a wide variety of investigations including physical and anthropometric examination, intelligence testing, full haematological study, chest X-ray, and psychiatric assessment.

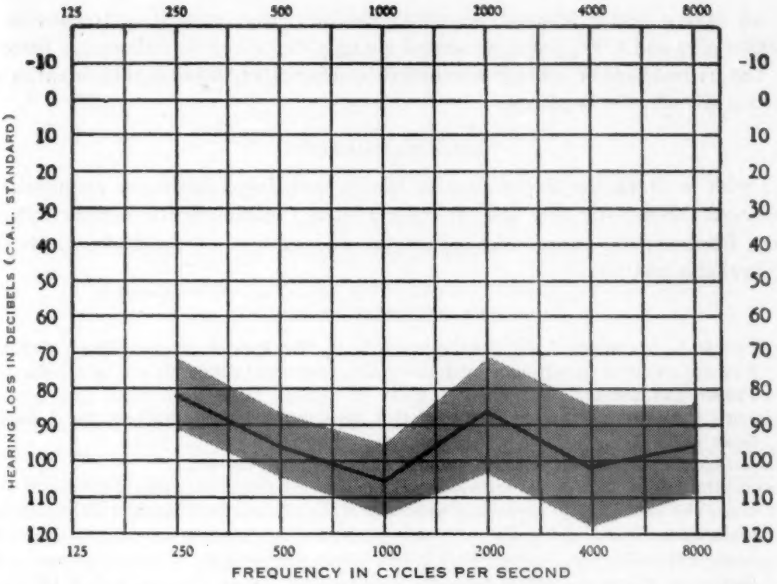
Special attention was given to other sex-linked traits such as colour vision, retinitis pigmentosa, haemophilia, etc. in an attempt to map the gene.

AUDIOGRAMS

Hearing tests showed a severe bilateral perceptive deafness, the loss in every patient being greater than 70 decibels for all frequencies. In particular it was noted that this was most severe for frequencies of 1,000 cycles and 4,000 cycles per second, and audiograms showed a pattern dipping at these levels in nine of the thirteen cases. A composite audiogram is illustrated (figure 2) which shows the characteristic pattern for this family group. The degree of deafness is so marked that no subject would derive any benefit from a hearing aid. All have very rudimentary speech development.

INTELLIGENCE

One patient (C-8, figure 1) is an imbecile and has been in a mental hospital for many years. His brother (C-6, figure 1) is a low grade moron and is being cared for in another government institution. The others were assessed on the performance items of the Wechsler-Bellevue Intelligence Test and, apart from the girl, achieved



Frequency in cycles per second	250	500	1000	2000	4000	8000
Mean Hearing Loss (In Decibels)	82	96	105	87	101	97
Standard Deviation	10.1	8.6	10.7	16.4	16.3	12.2

FIG. 2

an I. Q. ranging from 90 to 110. Her performance was hampered by nervousness and the resultant I. Q. figure of 84 must be considered a minimal estimate.

All but two of these patients, then, are potentially of average intelligence, but their deficient education and lack of emotional and social maturity prevent them from functioning at this level.

ASSOCIATED FEATURES

Vestibular function is normal, and no physical abnormalities have been found in association with this syndrome. None of the patients showed evidence of any of the known sex-linked traits. At first it was considered that the antisocial conduct found in three deaf members of the family was more than a coincidence, but detailed psychiatric investigation revealed environmental stress sufficient in each case to account for such behaviour. All deaf members of the group have made a poor social adjustment, and are satisfied with unskilled labouring jobs.

SUMMARY

Congenital deafness due to a sex-linked recessive gene is described in a family in which 15 members were affected.

All have a severe bilateral perceptive deafness most marked at frequencies of 1,000 cycles and 4,000 cycles per second giving a characteristic audiometric pattern. The patients are of average intelligence and no other physical abnormalities are associated with the syndrome.

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Huntington's Chorea in Michigan[1,2]

1. Demography and Genetics

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INTRODUCTION

AMONG THE RARE DOMINANT TRAITS of man, Huntington's chorea has enjoyed a certain notoriety ever since the well-known description of the disease by George Huntington in 1872. The striking and serious nature of the disease, including mental and emotional impairment as well as chorea, together with its dominant heredity, has attracted many investigators. The first recognition of the disease as a distinct entity may have been made by the Norwegian J. C. Lund in 1860 (cf. Ørbeck and Quelprud, 1954) but general knowledge of the disease only followed Huntington's paper in 1872. Among the most important genetic studies of this trait are those by Panse (1942), who made a study of the clinical, social, and genetic aspects of Huntington's chorea in the Rhineland, and by Bell (1934) and Sjögren (1935) who made critical studies of its formal genetics in material from the literature and from Swedish communities, respectively. Surveys of the frequency and distribution of the disease have now been made for several large areas, as will be summarized below. The many smaller studies will not be reviewed here.

The increased interest in the population genetics of man, particularly in natural selection and mutation, has focused attention on several ways in which Huntington's chorea appears to differ from most rare dominant traits. Family studies revealed that, in contrast to dominant traits such as for example, achondroplasia (Mørch, 1941) and neurofibromatosis (Crowe, *et al.*, 1955), propositi for Huntington's chorea almost invariably have a parent affected with Huntington's chorea if both parents were long-lived. The mutation rate therefore appears to be quite low. This finding

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agrees well with the high fertility of choreics (here and throughout this paper "choreic" refers only to Huntington's chorea), relative to their non-choreic sibs, found in several studies. In fact, the extensive study of Panse (1942) and the study of a single large family group by S. C. Reed and Palm (1951) both found choreics to be more fertile than their apparently normal sibs. If this were in fact the case, and there are a number of reservations which will be considered later, it would be of the greatest interest in human genetics. It would be an instance of a rare dominant gene in the process of replacing its "normal" allele. This process, postulated as part of the evolution of all organisms, has yet to be clearly demonstrated in man. [Neel (1957) has suggested that such a process may now be observable in Africa among the genes responsible for certain hemoglobin variants.] The unsatisfactory nature of the hypotheses needed to account for the present rarity of Huntington's chorea and the difficulty in imagining a population composed largely or solely of choreics add to the interest of this situation. The present investigation was undertaken as an attempt to elucidate this problem in population genetics, as well as to obtain other data, demographic and clinical, on Huntington's chorea in Michigan. A brief report of some of the results of this study has already been given (Reed, 1957).

METHOD OF STUDY

An attempt was made to obtain data on all persons known, or reliably reported, to have Huntington's chorea who have ever lived in the Lower Peninsula of Michigan (having 93.8 per cent of the total population of the state in 1940). For the purposes of the present study attention was primarily restricted to those kindreds (groups of biologically related individuals) having at least one medically diagnosed choreic living in the Lower Peninsula on April 1, 1940. This procedure was adopted for several reasons. One purpose of the study is to obtain frequency estimates and to make comparisons with U. S. Census data. Choreics usually do not come to medical attention for several years after onset of the disease, sometimes not for ten or more years. To be reasonably complete, a census of Huntington's chorea must be for some appropriate past date. April 1, 1940 was chosen as being suitable for completeness and also for coinciding with the U. S. Census of that year. Restricting the study to this date also has the advantage of making the data more homogeneous in time, an important consideration for certain variables of interest, e.g. fertility. For a few purposes only, such as the study of possible mutation, all available kindreds were utilized, and for other purposes, involving comparison within sibships, four sibships from the Upper Peninsula of Michigan, including five choreic members on April 1, 1940, were included.

Depending on the variable studied, data were available on choreics alone, choreics and their normal sibs, or choreics, normal sibs, and the population of Michigan as a whole. Comparison of choreics with the general population, as well as with normal sibs, should give valuable information not previously available.

This first part of the study will be concerned with the demography, social characteristics, and genetics of Huntington's chorea, while the second part will cover relative fitness and mutation. Clinical aspects of Huntington's chorea are not considered in this study but will be presented elsewhere.

ASCERTAINMENT AND EVALUATION OF THE DATA

The medically-diagnosed propositi of the kindred were obtained from several sources. Two trained field-workers, familiar with the disease, in 1954 and 1955 reviewed the diagnosis files of all State Hospitals (for mental patients) in Michigan and compiled a list of persons living or dead, with firm or possible diagnoses of Huntington's chorea. This list was deliberately made very inclusive so as to include unrecognized cases. The files of the Veterans Administration hospitals in Detroit and Battle Creek were also reviewed. Lists of all choreics seen in University Hospital of the University of Michigan, and Wayne County General Hospital (which serves Detroit largely), and a number of County Infirmaries were also obtained. Kindreds already on file in the Heredity Clinic, University of Michigan, were incorporated into the study. The variety of ways in which individuals were ascertained is illustrated in table 1. It may be noted that about one-third of the choreics living in 1940 were ascertained only through affected relatives, being unknown to official sources, while a little more than a third were ascertained through two or more independent sources.

The Michigan relatives of the new cases of definite or possible Huntington's chorea were contacted by one of the field workers and a detailed family history was obtained. When relatives were reported to have symptoms of chorea, whether in Michigan or not, these reports were checked as far as possible, by correspondence with relatives and hospitals if out of the state or, if in Michigan, by home visits and contacts with physicians and hospitals. Death certificates were obtained, when other information was inadequate, and were frequently of value. This follow-up

TABLE 1. METHOD OF ASCERTAINMENT OF WHITE CHOREICS RESIDENT IN THE LOWER PENINSULA OF MICHIGAN ON APRIL 1, 1940

Sex	Ever Institutionalized*	Mode of Ascertainment							Total
		1	2	3	4	5	6	7	
Male	Yes	1	4	20	0	0	23	6	54
	No	1	21	0	3	1	3	1	30
	Total	2	25	20	3	1	26	7	84
Female	Yes	0	2	26	0	0	38	9	75
	No	5	30	0	3	1	1	1	41
	Total	5	32	26	3	1	39	10	116
Total	Yes	1	6	46	0	0	61	15	129
	No	6	51	0	6	2	4	2	71
	Total	7	57	46	6	2	65	17	200

* As of 1956.

† 1: Only through a son or daughter.
2: Only through a choreic relative other than son or daughter.
3: Only through a mental institution (not County Infirmaries).
4: Only through University Hospital, Ann Arbor.
5: Only through other medical or social agencies.
6: Through any choreic relative *and* other sources.
7: Through two or more medical or social sources, not through choreic relatives.

procedure was carried out on each kindred, whether or not it appeared to contain a Michigan choreic living on April 1, 1940. A number of persons who had not been medically examined were examined at home or in University Hospital by one of us (J. H. C.) and a diagnosis of Huntington's chorea was made or excluded. After all indicated fieldwork, examinations, and correspondence had been completed, each kindred was reviewed for compatibility with a diagnosis of Huntington's chorea. Only those kindreds having one or more persons with a firm diagnosis were retained in the study. A number were removed because of inadequate diagnostic information. A supporting family history of chorea was not required for acceptance, but in the majority of kindreds this was present.

RESULTS

Race, sex, and age composition of the sample

The results to be given here are for the Lower Peninsula of Michigan for April 1, 1940 as described above. Two hundred and three individuals, either medically diagnosed or reliably reported to be choreic by lay sources (and having a close relative who is medically diagnosed) were ascertained. They were in 164 sibships which were in 124 kindreds. Three of these persons, in two sibships in separate kindreds, are of Negro ancestry; the others are of Caucasian ancestry. These 203 persons are divided between 85 males and 118 females. Of the 200 whites, 47 (23.5%) were in mental institutions on April 1, 1940. The above facts are presented in table 2 which gives distribution by age, sex, race, and institutional status. (In this and other tables the values for the mean, standard deviation, and standard error are computed from ungrouped data.)

In table 3 the choreic and diagnostic status on April 1, 1940 of members of sibships containing one or more persons with Huntington's chorea (according to medical

TABLE 2. AGE DISTRIBUTION OF WHITE* CHOREICS ON APRIL 1, 1940 IN THE LOWER PENINSULA OF MICHIGAN BY SEX AND INSTITUTIONAL STATUS

Sex	In Mental Institution	Age on April 1, 1940													No.	Mean	Standard Deviation	Standard Error
		15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79				
Male	Yes	0	0	1	1	1	4	2	2	3	1	4	1	0	20	52.35	12.86	2.88
	No	2	1	2	6	7	8	14	9	7	6	2	0	0	64	45.77	11.61	1.45
	Total	2	1	3	7	8	12	16	11	10	7	6	1	0	84	47.33	12.17	1.33
Female	Yes	0	0	0	0	1	5	7	3	4	4	2	0	1	27	52.59	9.84	1.89
	No	1	0	4	10	9	14	7	15	11	10	2	5	1	89	48.29	12.58	1.33
	Total	1	0	4	10	10	19	14	18	15	14	4	5	2	116	49.29	12.10	1.12
Total	Yes	0	0	1	1	2	9	9	5	7	5	6	1	1	47	52.49	11.10	1.62
	No	3	1	6	16	16	22	21	24	18	16	4	5	1	153	47.24	12.21	0.99
	Total	3	1	7	17	18	31	30	29	25	21	10	6	2	200	48.47	12.14	0.86

* Three Negro choreics were ascertained. Their status on April 1, 1940 was as follows: (1) Female, age 28, not institutionalized; (2) Female, age 29, institutionalized; (3) Male, age 51, institutionalized.

TABLE 3. STATUS OF MEMBERS OF SIBSHIPS CONTAINING ONE OR MORE CHOREICS ON APRIL 1, 1940. ONLY SIBSHIP MEMBERS LIVING IN THE LOWER PENINSULA OF MICHIGAN

Sex	Race	Choreic Status on April 1, 1940 and at Time of Last Investigation																Total
		1	1.5		2		3			4			5			6			
			A	B	A	B	A	B	C	A	B	C	A	B	C	A	B	C	
Male	White	22	1	0	61	47	3	1	0	100	8	1	0	0	0	8	2	2	195
	Negro	1	0	0	0	0	1	1	0	0	0	0	0	0	0	3	0	0	5
Female	White	25	3	3	88	65	0	0	0	114	13	4	2	0	0	2	1	1	234
	Negro	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	3
Total	White	47	4	3	149	112	3	1	0	214	21	5	2	0	0	10	3	3	429
	Negro	2	0	0	1	1	1	1	0	0	0	0	0	0	0	4	0	0	8

Status code: 1. Huntington's chorea diagnosed medically.
1.5 "Chorea" or ? Huntington's chorea diagnosed medically.
2. Huntington's chorea reported by reliable lay observers with no contradictions.
3. Huntington's chorea according to dubious or contradictory reports.
4. No Huntington's chorea according to lay report.
5. No Huntington's chorea according to medical report.
6. No information.
A = Status on April 1, 1940.
B = Number in A medically diagnosed between April 1, 1940 and June, 1956.
C = Number in A in status "2" at death or by June, 1956.

diagnosis or reliable, uncontradicted, lay report) is presented. (All lay reports were evaluated only with respect to choreiform movements.) Of the 203 white and Negro choreics, 49 had been medically diagnosed by April 1, 1940. Of the remaining 154 persons who are considered to have Huntington's chorea on that data (i.e. statuses 1.5 and 2 in table 3), 115 were medically diagnosed some time after April 1, 1940 but before June 1956. Two of the four dubiously choreic sibs (status 3, not counted as being choreic) developed definite Huntington's chorea at a later late, while 26 of the 216 non-choreic sibs (statuses 4 and 5) also later developed Huntington's chorea. It is thus possible to obtain some idea of the number of adults with the gene for Huntington's chorea who had not developed the disease by 1940. An estimate of the gene frequency will be presented later.

The 203 choreic individuals were ascertained from a population of 4,932,562 (Lower Peninsula of Michigan, 1940 U. S. Census), so that the observed frequency of choreics is 4.12×10^{-5} or about one choreic per 24,000 individuals. This is within the frequency range reported in other studies of populations of European ancestry, as shown in table 4, but, as indicated, none of these studies is exactly comparable to the present investigation. It does appear that the frequency in various populations of European origin (95.7 per cent of the Lower Peninsula is of European ancestry) is quite constant. In contrast to this "European" frequency range of $3.2–6.5 \times 10^{-5}$, the study of Kishimoto *et al.* (in press) in Japan (Aichi Prefecture, population 3,916,922) found a frequency of 3.22×10^{-6} (13 choreics). Since some 3,000 physicians in this prefecture are said to have been contacted regarding the possible

TABLE 4. FREQUENCY OF INDIVIDUALS WITH HUNTINGTON'S CHOREA IN CERTAIN AREAS*

Area Studied	Date of Census	Population of Area	Number of Choreics			Frequency of Choreics	Investigator	Remarks
			Male	Female	Total			
Rhineland, Germany	1933	7,690,266	—	—	248	3.22×10^{-5}	Panse (1942)	Retrospective frequency. Positive family history apparently required for each choreic
Northamptonshire, England	1955	263,000	5	12	17	6.5×10^{-5}	Pleydell (1954, 1955, 1957)	Not retrospective frequency
Minnesota, U. S. A.	1955	3,174,000 (estimated for 1955)	66	51	117	3.69×10^{-5}‡	Pearson *et al.* (1955) Pearson (1957)	Not retrospective frequency
Aichi Prefecture, Japan	1957	3,916,922	6	7	13	3.32×10^{-6}	Kishimoto *et al.* (in press)	Not retrospective frequency
Lower Peninsula of Michigan, U. S. A.	1940	4,932,562	85	118	203†	4.12×10^{-5}	Present study	Retrospective frequency

* A number of surveys are omitted because an intensive search for non-institutionalized choreics was not made.

† Includes 3 Negroes.

‡ 117/3,174,000. By estimating the number of choreics missed because of migration, Pearson *et al.* (1955) estimate the frequency of choreics in Minnesota to be 5.43×10^{-5}.

existence of Huntington's chorea among their patients, this low frequency may represent a real racial difference in frequency and not an example of under-reporting. If so, this difference raises interesting questions in population genetics concerning the relation of gene frequency and relative fitness in Japan and in the West. These subjects are considered further in the second part of this study.

The proportion of Negroes in Michigan in 1940 was 0.0422, while the observed proportion among the choreics is 0.0148. If the age-specific frequency of the trait is equally common in the two races, 8.314 Negro choreics would be expected. The observed number, three, does not differ significantly from this.

The sex distribution of the 203 choreics, 85 males and 118 females, can be shown to differ significantly from expectation. If the age-specific incidence of Huntington's chorea is equal in males and females, the expected number of choreic males is 106.058 (1940 U. S. Census age and sex data). The probability of deviations as great or greater than that observed is 0.004. The only other survey of a large area for which a sex distribution is given, that of Pearson *et al.* (1955) and Pearson (1957), reveals no deficiency of males, there being 66 males and 51 females. It is not clear why this deficiency of males in the present data should exist, especially since, as discussed

later, there appears to be no deficiency within sibships when considered as of the time of last investigation (i.e. using the status of each sibship member at time of death or as of June 1956). In the course of the study the impression was gained that there was a noticeably greater mobility of males, relative to females, in the early stages of the disease. If so this could lead to under-reporting during this period. The age distribution of table 2, however, does not give weight to this impression. On the other hand, the difference is not due to a sex difference in the mean duration of the disease (table 19).

Demographic and social characteristics

It is of interest to record the earliest known "origins" of the gene for Huntington's chorea present in the 124 kindreds of the Lower Peninsula in 1940. Some previous American studies (Davenport and Muncey, 1916; Vessie, 1932) have emphasized southeastern England as the area from which the choreic ancestors of American choreics came, although these authors recognize that other areas also contributed. The "origins" for the 124 kindreds in the Lower Peninsula are tabulated in table 5. Fifty-one kindreds could be traced outside of the United States; Canada, Germany, and England were the leading areas with 12, 11, and 7 kindreds, respectively. Fifty-two of 73 "American" kindreds were traced outside of Michigan, with New York leading by far with 19 kindreds. Without making a detailed investigation, it appears these "origins" are reasonably consistent with the patterns of immigration to the United States and the migrations leading to the present Michigan population.

The distribution of birthplaces of the white choreics living in 1940, listed in table 6, gives a more recent picture of migration. Twenty-seven (13.5%) of the choreics

TABLE 5. STATE (U. S. A.) OR FOREIGN COUNTRY OF ORIGIN OF EARLIEST TRACEABLE CHOREIC MEMBER OF 124 CHOREIC KINDREDS (LOWER PENINSULA OF MICHIGAN, 1940)

United States		Foreign Country	
State	Number	Country	Number
Michigan	21	Canada	12
New York	19	Germany	11
Indiana	7	England	7
Pennsylvania	7	Poland	4
Ohio	4	Ireland	3
Tennessee	3	Scotland	3
Illinois	2	Italy	2
Alabama	2	Netherlands	2
Arkansas	1	Austria	1
Connecticut	1	Czechoslovakia	1
Iowa	1	Finland	1
Kentucky	1	Greece	1
Maryland	1	Hungary	1
Massachusetts	1	Spain	1
Vermont	1	Yugoslavia	1
"New England"	1		
Total	73	Total	51

TABLE 6. PLACE OF BIRTH OF 200 WHITE CHOREICS LIVING IN THE LOWER PENINSULA OF MICHIGAN ON APRIL 1, 1940

State (U.S.A.) or Country of Birth	Male	Female	Total
Michigan	61	80	141
Indiana	2	5	7
Illinois	0	6	6
Ohio	1	2	3
New York	0	2	2
Other states	4	9	13
Uncertain	1	0	1
Total U. S. A.	69	104	173
Canada	6	5	11
Germany	1	3	4
Scotland	1	2	3
England	1	1	2
Austria	1	0	1
Czechoslovakia	1	0	1
Greece	1	0	1
Hungary	1	0	1
Netherlands	0	1	1
Poland	1	0	1
Spain	1	0	1
Total Foreign	15	12	27

were born outside of the United States, compared to 13.2 per cent in the whole population of the Lower Peninsula. Taking into consideration sex and difference in age composition between choreics and the general population, and assuming the same frequency of Huntington's chorea among immigrants as in native-born, we note that 15 (17.9% $\pm$ 4.2%) of the male choreics are foreign-born, compared to 21.63 expected from the age-specific census distribution, and 12 (10.3%$\pm$2.8%) of the female choreics are foreign-born, compared to 27.45 expected. The proportion of foreign-born males is lower than expected, but not significantly so, while the proportion of foreign-born females is also lower than expected, being significantly different from expected at the 0.001 level. It seems likely that this deficiency of foreign-born choreics reflects a tendency of foreign-born carriers of the gene for Huntington's chorea either (a) not to migrate (before showing the trait), or (b) after migrating not to be recognized as choreics, rather than a lower frequency of chorea in these countries. Data to decide this point are not available.

The residence of choreics in 1940, classified as urban or rural, and Wayne County (which includes Detroit) or not, is presented in table 7. As indicated, the definitions of "urban" and "rural" are those of the U. S. Census for that year. Of the choreics, 73.5 per cent had an urban address, compared to 66.6 per cent for the general population; the difference is not significant. There is no significant difference between observed and expected proportion of choreics living in the Detroit metropolitan area (included in Wayne County).

Information is available for 109 white choreics who were age 20 or more on April

TABLE 7. RESIDENCE OF WHITE CHOREICS LIVING IN THE LOWER PENINSULA OF MICHIGAN ON APRIL 1, 1940. (RESIDENCE OF PERSONS IN INSTITUTIONS IS THE LAST NON-INSTITUTIONAL ADDRESS.)

Group	In Mental Institutions	Urban or rural				Wayne County (incl. Detroit)[4]
		Urban[1]	Rural-non-farm[2]	Rural-farm[3]	Total	
Males	Yes	17	1	2	20	10
	No	50	6	8	64	23
	Total	67	7	10	84	33
Females	Yes	21	2	4	27	11
	No	59	14	16	89	24
	Total	80	16	20	116	35
Total choreics	Number	147	23	30	200	68
	Proportion of total	0.735	0.115	0.150	1.000	0.340
Population, Lower Peninsula, 1940 Census		0.666*	0.170*	0.164*	1.000*	0.392†

1. Cities and incorporated places of 2,500 or more inhabitants.
2. Not on farms or in urban areas.
3. All persons living on farms, regardless of occupation.
4. Wayne County in 1940 was 95.7 percent urban by these definitions.

* All races. Population was 4,932,562 in 1940, 95.7 percent of whom were Caucasians.

† White only. Population was 1,850,437 in 1940.

1, 1940 on the number of years of education completed. For 88 such choreics information is lacking. Comparable data on education for non-choreic sibs were not obtained. These data are given in table 8, together with the expected numbers calculated from the 1940 census data for Michigan. If one considers only two educational groups, 0–6 years, and 7 or more years, and pools age groups (which do not differ significantly), it is found that the distribution of males differs significantly ($P < 0.01$) from expected, there being an excess in the 0–6 class, while females do not differ from expectation. Also, male choreics differ from female choreics ($P < 0.01$), there again being an excess of males in the 0–6 class. Since reliable educational data are available only for 55 per cent of the choreics, it is not clear how much significance to give these findings. It does appear that there is a sex difference in the amount of education received. Since this difference occurred before the onset of choreic movements, and, in many cases, before other signs of Huntington's chorea have appeared, the question arises whether this difference is due to some pre-choreic influence of the gene on its bearer or to social environment, which usually includes a choreic parent. Some children will have had to go to work early because their father was incapacitated by Huntington's chorea.

The distribution of "pre-choreic" occupations of choreics is of interest insofar as occupation is an indicator of social and intellectual competence. It is obvious that if significant differences are found they could be due to an early effect of the gene on its bearer or to the fact that a parent or other relative had Huntington's chorea.

TABLE 8. YEARS OF EDUCATION COMPLETED BY 109 WHITE CHOREICS IN THE LOWER PENINSULA OF MICHIGAN, AGE 20 YEARS OR MORE ON APRIL 1, 1940. (EXPECTED NUMBER, CALCULATED FROM 1940 CENSUS FOR MICHIGAN*, GIVEN IN PARENTHESES.)

Sex	Age	Years of education			Total	Education Unknown
		0-6	7-12	13 or More		
Males	20–44	6	11	1	18	13
		(1.7)	(14.2)	(2.1)	(18.0)	
	45 and over	11	14	0	25	26
		(8.5)	(14.9)	(1.6)	(25.0)	
	Total	17	25	1	43	39
		(8.4)	(30.4)	(4.2)	(43.0)	
Females	20–44	2	27	3	32	11
		(2.7)	(25.6)	(3.7)	(32.0)	
	45 and over	6	25	3	34	38
		(9.7)	(22.2)	(2.1)	(34.0)	
	Total	8	52	6	66	49
		(10.7)	(49.0)	(6.3)	(66.0)	
Males and females	20–44	8	38	4	50	24
		(4.5)	(39.6)	(5.9)	(50.0)	
	45 and over	17	39	3	59	64
		(18.4)	(36.8)	(3.8)	(59.0)	
	Total	25	77	7	109	88
		(19.5)	(79.0)	(10.5)	(109.0)	

* For all races, native and foreign-born.

Most studies have found that choreics appear to be members of the lower socio-economic levels of society. Panse (1942), for example, in his survey of the Rhineland, found 1.1 per cent of choreics were in the class of high officials and university teachers, while 21.3 per cent were unskilled laborers. The corresponding figures for his "comparison" kindreds (normal descendants of normal sibs of choreics) were 2.7 per cent and 13.4 per cent. These figures differ significantly for unskilled laborers. Panse did not have census data for comparison but data from several German studies also suggested that choreics and their normal sibs average lower on the social and occupational scale. The relevant comparison of occupations of choreics, their normal sibs, and the 1940 census, in the present study, is difficult for several reasons: (1) The "best" occupation of choreics seems most useful, since, as of 1940, most will be severely affected by the disease, but this occupation will occur at different times. (2) Decisions must sometimes be made as to which occupation is "best." (3) The normal sibs may not have their "best" occupation in 1940. (4) The major occupational groups of the 1940 census, used for classifying, are often internally heterogeneous. (5) For married women, the occupation of the 1940 husband must be used. For the comparison of occupations all sibships containing a choreic living on April 1, 1940 were used, but the choreic status of sibship members was taken as of the time of last investigation, i.e. at death or as of June 1956. Thus there are some choreics included who were "normal" on April 1, 1940. When a person had more than one major occupational group, the "best" one, according to the ranking shown in table 9, was used for tabulating. This ranking is unavoidably arbitrary.

TABLE 9. DISTRIBUTION OF "BEST"* OCCUPATIONS OF CHOREICS AND THEIR NON-CHOREIC SIBS. SIBSHIPS CONTAINING ONE OR MORE CHOREICS ON APRIL 1, 1940 IN MICHIGAN (LOWER PENINSULA PLUS FOUR UPPER PENINSULA SIBSHIPS). CHOREIC STATUS AS OF THE TIME OF LAST INVESTIGATION (DEATH OR JUNE 1956). NUMBERS IN PARENTHESES ARE NUMBERS OF PERSONS WITH TWO OR MORE OCCUPATIONS. PROPORTIONS ARE GIVEN BELOW THE NUMBERS AND ARE BASED ON TOTAL KNOWN.

Choreic Status	Major Occupational Group (1940 Census classification)									Total Known	Unknown	Total
	1	2	3	4	5	6	7	8	9			
Male												
Choreic	2 (0) .0156	13 (7) .1016	27 (4) .2109	10 (2) .0781	15 (6) .1172	40 (8) .3125	10 (3) .0781	3 (1) .0234	8 (0) .0625	128 0.9999	12‡	140
Doubtfully choreic	1 (0) —	0	2 (1) —	0	1 (1) —	2 (0) —	1 (0) —	1 (1) —	2 (0) —	10 —	2	12
Non-choreic	5 (1) .0490	15 (2) .1471	18 (0) .1765	4 (2) .0392	15 (3) .1471	31 (3) .3039	3 (0) .0294	1 (0) .0098	10 (0) .0980	102 1.0000	19	121
Unknown	0	0	0	0	0	1 (0) —	0	1 (0) —	0	2 —	2	4
Proportion§ in Michigan	0.0584	0.1003	0.2066	0.1160	0.1117	0.2453	0.0580	0.0264	0.0772	0.9999	—	—
Female												
Choreic (married†)	3 (1) .0256	13 (2) .1111	30 (5) .2564	6 (2) .0513	26 (3) .2222	15 (1) .1282	4 (0) .0342	2 (0) .0171	18 (0) .1538	117 0.9999	40	157
Choreic (single)	6 (3) .3333	1 (0) .0556	1 (1) .0556	3 (1) .1667	0	1 (0) .0556	6 (0) .3333	0	0	18 1.0001	0	18
Doubtfully choreic (married†, no single)	0	1 (0) —	0	1 (0) —	1 (0) —	1 (0) —	0	0	0	4 —	3	7
Non-choreic (married†)	2 (0) .0339	11 (3) .1864	9 (1) .1525	5 (1) .0847	11 (1) .1864	16 (2) .2712	1 (1) 0.169	0	4 (0) .0678	59 0.9998	51	110
Non-choreic (single)	5 (2) .2632	2 (0) .1053	0	2 (0) .1053	0	5 (0) .2632	5 (0) .2632	0	0	19 1.0002	7	26
Unknown (married†, no single)	0	0	0	1 (1) —	0	0	0	0	0	1 —	1	2
Proportion§ in Michigan	0.1641	0.0505	0.0156	0.2992	0.0118	0.1665	0.2748	0.0043	0.0133	1.0001	—	—

Major occupational groups:

1. Professional and semi-professional workers.
2. Proprietors, managers, and officials, except farm.
3. Craftsmen, foremen, and kindred workers.
4. Clerical, sales, and kindred workers.
5. Farmers and farm managers.
6. Operatives and kindred workers (manual, usually routine, requiring little or no training).
7. Service workers (domestics, policemen, janitors, waitresses, etc.)
8. Farm laborers and foremen.
9. Laborers, except farm and mine.

* "Best" occupation as of time of last investigation. When a person had two or more major occupational groups over a period of time, the one with the lowest column number equivalent (i.e., 2 = proprietors, etc.) was chosen for tabulation.

† Occupation of *husband*.

‡ Includes one choreic who was "unemployed and seeking work."

§ Data from 1940 census for Michigan for persons 25 or more years of age with known occupation.

TABLE 10. POOLED DISTRIBUTION OF "BEST" OCCUPATIONS, CONDENSED FROM TABLE 9. NUMBER AND PROPORTION IN EACH CATEGORY.

Sex	Choreic status	Pooled major occupational groups* 1 + 2	3 + 4 + 5	6 + 7 + 8 + 9	Total
Males	Choreic	15	52	61	128
		0.1172	0.4062	0.4765	0.9999
	Non-choreic	20	37	45	102
		0.1961	0.3628	0.4411	1.0000
Married females	Choreic	16	62	39	117
		0.1367	0.5299	0.3333	0.9999
	Non-choreic	13	25	21	59
		0.2203	0.4236	0.3559	0.9998
Proportion of males in Michigan, 1940		0.1587	0.4343	0.4069	0.9999

* For married females, occupation of husband is used.

The distribution of "best" occupations is given in tables 9 and 10. Although table 9 contains too many categories for convenient use, it reveals that (a) choreic males and (b) husbands of choreic females are significantly deficient in professional and semi-professional workers (2-tailed probabilities less than 0.001 and 0.02, respectively) compared with the state of Michigan in 1940. On the other hand, the proportion of laborers does not differ significantly. Testing the distributions of grouped occupa-

TABLE 11. MARITAL STATUS ON APRIL 1, 1940 OF CHOREICS AND THEIR NON-CHOREIC SIBS LIVING IN MICHIGAN.* NUMBER FOR EACH CATEGORY. PERSONS WITH UNKNOWN MARITAL STATUS OMITTED (26). PROPORTION IN MICHIGAN FROM 1940 CENSUS.

Sex-Group-Institutional Status	Age 15–29 years					Age 30–44 Years					Age 45 or More Years				
	Single	Married	Divorced	Widowed	Total	Single	Married	Divorced	Widowed	Total	Single	Married	Divorced	Widowed	Total
Male choreic															
In	1	0	0	0	1	1	3	2	0	6	2	5	3	0	10
Out	3	2	0	0	5	5	12	3	0	20	7	24	3	4	38
Total	4	2	0	0	6	6	15	5	0	26	9	29	6	4	48
Female choreic															
In	0	0	0	0	0	1	4	1	1	6	2	12	4	6	24
Out	3	4	1	0	8	1	29	3	1	34	3	35	2	8	48
Total	3	4	1	0	8	2	33	4	1	40	5	47	6	14	72
Male non-choreic sibs	3	9	1	0	13	4	19	1	0	24	7	33	3	2	45
Female non-choreic sibs	4	9	0	0	13	2	22	1	0	25	9	33	1	5	48
Male-state population	.6975	.2969	.0044	.0012	—	.1545	.8124	.0211	.0120	—	.1092	.7616	.0242	.1049	—
Female-state population	.5241	.4627	.0095	.0038	—	.0882	.8492	.0287	.0340	—	.0651	.6626	.0198	.2525	—

* Lower Peninsula plus four Upper Peninsula sibships.

tions of table 10 shows that there are no significant differences between choreic males and non-choreic males or between males in the general population, similarly for husbands of choreic females. Pooling male choreics and husbands of female choreics to make a general class of choreics, (A), and male non-choreics and husbands of female non-choreics to make a class of non-choreics, (B), reveals no difference between Michigan males and A or B. However, A and B differ at the 0.05 level if occupations 1 and 2 are pooled to make one class and 3 through 9 another (but not for table 10 as it stands). All of these tests are selected out of a very large number of possible comparisons and their true significance is somewhat questionable. There is a definite suggestion, however, that choreics have a lower occupational status than their non-choreic sibs or the general population, possibly due more to a relative deficiency of "higher" occupations than to an excess of "lower" occupations. On the other hand, the choreics do not differ grossly from these two groups and the non-choreic sibs do not differ significantly from the general population. This absence of major differences is perhaps the clearest result.

The marital status of choreics and their non-choreic sibs on April 1, 1940 is presented in table 11 and the total number of marriages by that date is given in table 12. Table 11 illustrates the observed tendency of choreics to be divorced by their normal spouses before or during the time of institutionalization. Neither the choreics nor their non-choreic sibs differ significantly from the general population (comparisons being made within sexes). In the two higher age groups the proportion of single male choreics exceeds the corresponding proportion among female choreics and, if these two age groups are pooled, the proportions are found to differ at the 0.01 level of significance. It is interesting that Panse (1942) also found that the proportion of male choreics, age 30 or over, who were single (27.9% ± 2.9%) was significantly higher than the corresponding proportion for female choreics (17.8% ± 2.5%).

TABLE 12. NUMBER OF MARRIAGES BY APRIL 1, 1940 OF CHOREICS AND NON-CHOREIC SIBS. LOWER PENINSULA OF MICHIGAN PLUS FOUR UPPER PENINSULA SIBSHIPS.

Sex-Group-Institutional Status	15–29 Years of Age						30–44 Years of Age						45 or More Years of Age						
	Number				Total persons	Mean	Number				Total persons	Mean ± S.E.	Number					Total persons	Mean ± S.E.
	0	1	2	3			0	1	2	3			0	1	2	3	4		
Male choreic																			
In	1	0	0	0	1	0	1	3	2	0	6	1.17±0.31	2	9	1	0	0	12	0.92±0.15
Out	3	2	0	0	5	0.04	5	16	0	0	21	0.76±0.10	7	28	2	1	0	38	0.92±0.10
Total	4	2	0	0	6	0.33	6	19	2	0	27	0.85±0.10	9	37	3	1	0	50	0.92±0.08
Female choreic																			
In	0	0	0	0	0	0	1	5	0	0	6	0.83±0.17	2	17	4	2	0	25	1.24±0.14
Out	3	4	1	0	8	0.75	1	25	7	1	34	1.24±0.09	3	38	7	1	1	50	1.18±0.09
Total	3	4	1	0	8	0.75	2	30	7	1	40	1.18±0.09	5	55	11	3	1	75	1.20±0.08
Male non-choreic	3	10	0	0	13	0.77	4	19	2	0	25	0.92±0.10	7	37	3	0	0	47	0.91±0.07
Female non-choreic	4	9	0	0	13	0.69	2	22	1	0	25	0.96±0.07	9	38	6	0	0	53	0.94±0.07

TABLE 13. DISTRIBUTION OF INDIVIDUALS WHO HAD CHILDREN BORN OUT OF WEDLOCK. WHITE SIBSHIPS AT TIME OF LAST INVESTIGATION (DEATH OR JUNE 1956). LOWER PENINSULA OF MICHIGAN PLUS FOUR UPPER PENINSULA SIBSHIPS. INDIVIDUALS AGE 20 OR MORE WHO ARE TRACEABLE.

Sex	Choreic status	Number who had children born out of wedlock	Total number traceable
Male	Choreic	2	162
	Doubtfully choreic	0	15
	Non-choreic	1	155
	Unknown	0	9
Female	Choreic	8	197
	Doubtfully choreic	0	14
	Non-choreic	1	179
	Unknown	0	7

The non-choreic male sibs do not differ significantly from the non-choreic female sibs. None of the pooled age groups can be shown to differ from the general population. Inspection of table 12 reveals a definite tendency of female choreics, relative to other groups, to have several marriages. Confining attention to choreics who were not institutionalized on April 1, 1940 and comparing mean number of marriages, female choreics age 30–44 years are seen to differ significantly from male choreics ($P < 0.001$) and from female non-choreic sibs ($P < 0.02$) of the same age group. For the 45 and over age group, female choreics do not differ significantly from male choreics (but again have a higher mean number of marriages) yet still differ from female non-choreics ($P < 0.05$). Male choreics do not differ significantly from male non-choreics in these two age groups. Census data for this variable are not available. In order to interpret these findings properly a knowledge of the time of marriage in relation to the onset of Huntington's chorea is necessary, and this is not available. It is safe to assume, however, that almost all marriages take place before manifestation of choreiform movements has begun, though not necessarily before emotional or mental changes. The increased marriage rate observed in female choreics does appear to reflect an effect on its bearer of the gene for Huntington's chorea.

Table 13 gives the distribution, by choreic status, of individuals who have had children born out of wedlock. Male choreics do not differ from male non-choreic sibs or female non-choreic sibs, but female choreics differ significantly ($P < 0.05$, exact 2 x 2 test) from their female non-choreic sibs, 8 out of 197 having had an illegitimate child. This finding may well be a direct consequence of their chorea since, of the five whose ages at the time of onset of choreic movements are known, three had their illegitimate child after onset of choreic movements.

An attempt was made to compare criminal behavior of choreics and their non-choreic sibs. Through the kind cooperation of Commissioner Joseph A. Childs it was possible to check the files of the Michigan State Police for the names of sibship members and obtain details of crimes on record. For this comparison only sibship members, choreic and non-choreic, known to be living in Michigan on April 1, 1940, and also at the time of last investigation, were checked. The results are given in table 14. Five of 103 male choreics were recorded as having committed a serious crime, compared to none of 87 male non-choreics. These proportions differ signifi-

cantly at the 0.05 level. Three of 141 female choreics were similarly recorded, compared to none of 98 female non-choreics. These proportions do not differ significantly. For both sexes, two of the three individuals whose choreic status was known at the time of their "major" crime were choreic then (i.e. they had choreic movements). There may have been an increase in criminal activity of persons having the gene but not yet showing chorea, but these data do not prove it.

In reviewing these data on social and demographic characteristics of choreics and their normal sibs, perhaps the most noteworthy finding is that it was not possible to show that unaffected sibs differed from the general population with regard to occupational and marital status. These comparisons are not particularly suited for our purposes but it does appear that there are no marked differences. Thus, the distribution of occupations, for example, indicates that choreic kindreds, in the absence of Huntington's chorea, would not be clearly distinguished from the general population. It is difficult to believe, however, that the presence of affected individuals in a kindred for several generations would have no effect on the social behavior and economic status of unaffected members of the kindred. Our negative finding is contrary to that of most authors, but, of these, only Panse (1942) has made a large, comparative survey. It is not surprising that choreics are found to differ from non-choreic sibs and from the general population, but some of the differences between choreic males and choreic females are harder to explain. Any interpretation of differences between choreics and the general population is complicated by the facts that a given social action might reflect (a) the presence of the gene on its bearer or (b) the social environment created by choreic relatives. If due to (a) one can further inquire whether this effect may precede the physical symptoms of chorea. Since choreics and

TABLE 14. CRIMES COMMITTED BY SIBSHIP MEMBERS ON RECORD WITH MICHIGAN STATE POLICE. ONLY INDIVIDUALS LIVING IN MICHIGAN ON APRIL 1, 1940 AND ALSO AT TIME OF LAST INVESTIGATION (DEATH OR JUNE 1956).

Sex	Choreic status at: Last investigation	Choreic status at: Time of crime	Individuals having recorded crimes* Category 1	2	3	Total	Individuals having no recorded crimes
Male	Choreic	Choreic	2	0	2	4	
		Non-choreic	0	1	2	3	94
		Unknown	1	1	0	2	
	Non-choreic	—	0	0	2	2	85
	Unknown	Unknown	0	0	2	2	10
Female	Choreic	Choreic	0	2	0	2	
		Non-choreic	0	1	0	1	138
		Unknown	0	0	0	0	
	Non-choreic	—	0	0	0	0	95
	Unknown	Unknown	0	0	0	0	5

* When an individual committed crimes in more than one category, the more serious category was tabulated.

Crime categories: 1. Murder, accomplice to murder, rape.
2. Breaking and entering, larceny, false pretenses, soliciting for prostitution.
3. Drunkeness, illegal possession of liquor, traffic violations, other minor charges.

their non-choreic sibs have had relatively similar early social environments, those traits for which choreics differ from the general population, and non-choreics do not, can be said to be due to possibility (a). This is the case for occupation. It is not possible to state what proportions of the differences found are due to mental and emotional changes before onset of chorea. The "best" major occupational group and the ultimate marital status, in many cases, have been determined by the age at which chorea usually develops, but certainly not in all cases. After onset of chorea, of course, it is hardly remarkable that differences should be found. The data on illegitimacy and criminality appear to demonstrate differences of this sort. It therefore seems difficult and perhaps unjustified, to go beyond the obvious conclusion that overt chorea affects social and intellectual behavior.

PROGRESS OF THE DISEASE

Although the present study is not primarily concerned with the clinical aspects of Huntington's chorea, certain data on the characteristics of the disease are necessary to the arguments to be developed later in this and a paper to be published. Objective data on three landmarks in the progress of the disease, *onset*, *institutionalization*, and *death*, are available and will be considered in this section. Data on suicide and cause of death will also be presented.

The age at onset is the most important statistic to be considered and it is necessary to emphasize some unavoidable deficiencies in this variable. We are concerned here with the onset of choreiform movements, not of mental or emotional changes, since this is the only aspect for which reasonably objective data, for both institutionalized and uninstitutionalized individuals, can be obtained. In any individual instance, however, the age at onset may be in error by several years, since discrepancies of this order between reports of equally reliable observers, close to the choreic concerned, are quite common. When the reports differed by more than several years the age was considered to be unknown, and for some persons it was not possible to obtain any

TABLE 15. AGE AT ONSET OF CHOREIC MOVEMENTS. STATUS AT TIME OF LAST INVESTIGATION (DEATH OR JUNE 1956). INDIVIDUALS WITH KNOWN AGE OF ONSET IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940.

Sex	Living or Dead	Age at Onset										Total	Mean	Standard Deviation	Standard Error
		15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69				
Male	Living	2	2	2	5	8	2	2	0	0	0	0	23	33.70	8.36	1.74
	Dead	4	7	9	9	13	10	6	4	0	0	1	63	34.86	10.28	1.30
	Total	6	9	11	14	21	12	8	4	0	0	1	86	34.55	9.77	1.05
Female	Living	1	5	1	6	3	6	2	2	0	0	1	27	35.93	11.79	2.27
	Dead	5	8	8	15	18	21	12	4	0	0	0	91	35.82	9.24	0.97
	Total	6	13	9	21	21	27	14	6	0	0	1	118	35.85	9.83	0.90
Male and Female	Living	3	7	3	11	11	8	4	2	0	0	1	50	34.90	10.32	1.46
	Dead	9	15	17	24	31	31	18	8	0	0	1	154	35.43	9.66	0.78
	Total	12	22	20	35	42	39	22	10	0	0	2	204	35.30	9.80	0.69

information on onset. Other workers have also remarked on the difficulties of determining the age at onset.

All data on the progress of the disease pertain to the choreic members of sibships which, on April 1, 1940, contained one or more choreic individuals living in Michigan. Where applicable, e.g., for age at death, data on these individuals as of the time of last investigation is used. This procedure will make the data more homogeneous in time than they would be if other sibships and generations were used; for variables such as age at institutionalization and age at death, such homogeneity is clearly desirable. It is believed that the onset data are also improved. This restriction in time introduces some bias, since there is selection for a long onset-death interval in the sibship propositus, but this seems less serious than the disadvantage of data covering a wider time span. The choreic sibs of the 1940 propositus, of course, are not selected for long onset-death interval.

In table 15 the distribution of age at onset in 204 choreics is presented. None of the divisions by sex or by whether living or dead differ significantly in mean or variance so that the value for the total distribution may be used, the mean being 35.30 ± 0.69 years, standard deviation 9.80 years. This mean agrees closely with that found in other large studies, e.g. Panse (1942): 36.19 years; Bell (1934): 35.51 years. The standard deviation is significantly (at the 0.001 level) smaller however. In the two studies above, it was 12.3 and 12.38 years, respectively. In keeping with the smaller standard deviation of the present study, the range of ages is less, being 15 to 65, while both Panse (446 cases) and Bell (460 cases) report individuals in the 0–4 and the 70–74 age groups. In comparing these studies it is necessary to remember that Panse's data cover several generations, including persons long-deceased and Bell's are compiled from the literature and are therefore somewhat selected. It is not clear how real this difference in standard deviation is.

The distribution of the age at first institutionalization (because of Huntington's chorea) is presented in table 16. The mean for 89 males is 48.11 ± 1.22 years; for 103 females it is 48.87 ± 1.13 years. There appear to be no data, for a specified area, for comparison and, in fact, this variable, by itself, is too much a function of local hospital facilities and social customs to be valuable for comparisons.

TABLE 16. AGE AT FIRST INSTITUTIONALIZATION. STATUS AT TIME OF LAST INVESTIGATION (DEATH OR JUNE 1956). INDIVIDUALS EVER INSTITUTIONALIZED IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940.

Sex	Living or Dead	Age at First Institutionalization											Total	Mean	Standard Deviation	Standard Error
		20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79				
Male	Living	0	0	1	4	4	2	1	2	1	0	0	1	16	46.38	11.59	2.90
	Dead	0	3	5	10	11	10	9	12	5	7	0	1	73	48.49	11.51	1.35
	Total	0	3	6	14	15	12	10	14	6	7	0	2	89	48.11	11.48	1.22
Female	Living	0	0	1	5	3	5	4	3	1	3	0	1	26	49.92	11.76	2.31
	Dead	1	1	6	8	16	11	12	5	10	4	2	1	77	48.52	11.40	1.30
	Total	1	1	7	13	19	16	16	8	11	7	2	2	103	48.87	11.45	1.13

TABLE 17. AGE AT DEATH OF CHOREIC INDIVIDUALS. INDIVIDUALS IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940. S.D. = STANDARD DEVIATION, S.E. = STANDARD ERROR.

Sex	Age at death																	
	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	Total	Mean	S.D.	S.E.
Male	1	2	4	12	14	15	23	12	16	12	8	5	1	0	125	53.05	12.65	1.13
Female	1	2	5	8	10	23	25	17	18	13	9	5	0	1	137	54.11	12.29	1.05

The age of choreics at death has been studied by a number of workers. In table 17 the distribution of these ages for 125 males and 137 females is given. The respective means of 53.05 ± 1.13 and 54.11 ± 1.05 years are strikingly close to those reported by Bell (1934) and Panse (1942). The former gives 53.55 ± 0.63 and 52.59 ± 0.74 years and the latter 52.24 ± 0.76 and 52.15 ± 0.69 years, respectively. The deaths in the present study include eight "unnatural" deaths (accidents and suicides) among males and five among females. For comparison, the age at death in 1940 for whites of the death-registration states of the U. S. was 62.1 years for males and 66.6 years for females (Vital Statistics—Special Reports, Vol. 33—U. S. Public Health Service). The absence of a significant sex difference is not surprising if the chorea is a fairly direct cause of death, overriding to an appreciable extent other causes of death. The definite effect of the disease on life span is seen more clearly in the distribution of the onset-death interval given below.

The distribution of the interval between onset and first institutionalization, for 49 males and 69 females, is given in table 18. The mean for males is 10.59 ± 0.96 years and for females, 12.67 ± 0.98 years. These means do not differ significantly. Since the propositus for each kindred is ascertained after he has come to medical attention, the distribution in table 18 indicates that any survey of choreics is likely to miss a certain proportion of affected individuals. This proportion should be small in the present study since kindreds were ascertained through all known choreics in Michigan, including long-deceased individuals, but if a 1940 kindred were represented by only one living Michigan choreic, and by no deceased Michigan choreics, a long onset-institutionalization interval could lead to non-ascertainment.

The interval between onset and death is a good indicator of the progress of the disease. The distribution of this variable for 65 males and 88 females is given in table 19. The mean for males is 15.78 ± 1.00 years; for females it is 15.93 ± 0.91 years. The corresponding figures found by Bell (1934) are 13.72 years (sexes pooled)

TABLE 18. INTERVAL BETWEEN ONSET AND FIRST INSTITUTIONALIZATION OF CHOREIC INDIVIDUALS. INSTITUTIONALIZED INDIVIDUALS IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940.

Sex	(Year of First Institutionalization)—(Year of Onset)								Total	Mean	Standard Deviation	Standard Error
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	48				
Male	9	14	13	8	3	1	1	0	49	10.59	6.75	0.96
Female	10	19	13	12	12	1	1	1	69	12.67	8.16	0.98

TABLE 19. INTERVAL BETWEEN ONSET AND DEATH OF CHOREIC INDIVIDUAL. INDIVIDUALS IN SIBSHIP CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940.

Sex	Age of Onset	(Year of Death)—(Year of Onset)									Total	Mean	Standard Deviation	Standard Error	Average Future Life-time—U.S. White Population, 1940*
		0–4	5–9	10–14	15–19	20–24	25–29	30–34	36	45	51					
Males	15–19	0	0	1	3	0	0	0	0	0	0	4	15.25	2.06	1.03	50.5
	20–24	1	1	4	0	0	0	1	0	0	0	7	15.57	9.18	3.47	46.0
	25–29	0	1	2	4	1	0	0	1	1	0	10	20.20	11.87	3.75	41.5
	30–34	1	1	2	5	1	1	0	0	0	0	11	15.00	7.18	2.17	37.0
	35–39	2	0	2	7	0	0	2	0	0	0	13	15.77	9.07	2.51	32.6
	40–44	0	1	4	3	1	1	0	0	0	0	10	15.60	5.93	1.87	28.3
	45–49	1	2	0	3	0	0	0	0	0	0	6	11.83	7.20	2.94	24.3
	50–54	0	0	3	1	0	0	0	0	0	0	4	14.25	2.06	1.03	20.5
	Total	5	6	18	26	3	2	3	1	1	0	65	15.78	8.09	1.00	—
Females	15–19	0	0	1	1	2	0	0	0	0	1	5	24.60	15.74	7.04	54.2
	20–24	1	1	2	0	1	0	1	0	0	0	6	14.50	11.11	4.54	49.5
	25–29	0	1	1	0	3	3	0	0	0	0	8	20.50	6.72	2.38	45.0
	30–34	1	3	2	6	3	0	0	0	0	0	15	13.87	6.32	1.63	40.4
	35–39	0	3	5	6	1	1	2	0	0	0	18	16.61	7.43	1.75	35.9
	40–44	1	5	4	5	1	3	0	0	0	0	19	14.32	7.81	1.79	31.5
	45–49	1	0	5	3	1	1	1	0	0	0	12	16.33	8.72	2.52	27.2
	50–54	1	0	1	2	0	0	0	0	0	0	4	11.75	7.27	3.64	23.1
	55–59	0	0	0	0	0	0	0	0	0	0	0	—	—	—	19.2
	60–64	0	0	0	0	0	0	0	0	0	0	0	—	—	—	15.6
	65+	0	1	0	0	0	0	0	0	0	0	1	6.00	—	—	—
	Total	5	14	21	23	12	8	4	0	0	1	88	15.93	8.55	0.91	—

* Years of life remaining for normal individuals in indicated group. Data for mid-age of the five year interval, e.g., 17, 22, etc. (Vital Statistics—Special Reports, U. S. Bureau of the Census, 1945).

and by Panse (1942) 13.87 ± 0.62 and 13.05 ± 0.48 years. The present data agree with those of Bell in not finding a clear effect of age of onset on the interval between onset and death. Comparison in table 19 of the onset-death intervals with the expected future survival in normal individuals reveals a definite shortening of life in the choreics, especially among those with early onset.

An increased rate of suicide among choreics has been noted by many authors and the data of table 20 also show this tendency, the frequency among non-institutionalized male choreics being significantly elevated relative to male non-choreics and similarly for females. Suicide was an important cause of death among the non-institutionalized choreic population, accounting for 7.8 per cent of deaths in males and 6.4 per cent in females (sibship data).

Cause of death is often difficult to assign and, when several contributing factors occur together, is often difficult to interpret, but it may still be of interest to list some major categories of causes of death of choreics who died after April 1, 1940. Table 21 presents these data for 13 deaths under age 40 and 148 deaths at age 40 or over. Aside from Huntington's chorea, heart disease and pneumonia are most frequently listed among the causes of death. The former cause is not unusual in any population mostly over age 40 but the latter seems to be a more particular complica-

TABLE 20. DISTRIBUTION OF SUICIDES AMONG INDIVIDUALS IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940. ONLY INDIVIDUALS SURVIVING TO AGE 15 OR OLDER. THREE INDIVIDUALS REPORTED NORMAL BUT HAVING CHILDREN WITH MEDICALLY DIAGNOSED HUNTINGTON'S CHOREA ARE OMITTED. STATUS AT TIME OF LAST INVESTIGATION.

Individuals	In Mental Institution	Total Number of Individuals	Number of Suicides	Proportion of Individuals Committing Suicide	Range of Ages at Time of Suicide
Male choreics	Yes	91	0	—	
	No	77	6	0.078	37–50
	Total	168	6	0.036	
Female choreics	Yes	105	0	—	
	No	94	6	0.064	30–78
	Total	199	6	0.030	
Male non-choreics		161	2	0.012	39–42
Female non-choreics		181	1	0.006	39

TABLE 21. CAUSE OF DEATH OF WHITE CHOREICS ALIVE IN MICHIGAN ON APRIL 1, 1940. DATA FROM MEDICAL RECORDS, EXCEPT CONCERNING ACCIDENTS AND SUICIDES. "H. C." = HUNTINGTON'S CHOREA

Recorded Cause	Age at Death: Under 40	Age at Death: 40 or over
H. C. only	4	14
H. C. and one or more other diseases	2	42
No H. C. but one or more other diseases	2	42
Accident or suicide	2	1
Not given	3	49
Total	13	148
Causes below counted irrespective of other concomitant diseases:		
Heart disease, any form	0	23
Pneumonia, any form	3	22
Exhaustion, debility or senility	0	6

tion of chorea, largely in consequence of the terminal debility and the aspiration of food into the lungs. Perhaps the most striking fact is that of five choreics dying under 40 whose cause of death is reported and is not due solely to Huntington's chorea, accident, or suicide, three are reported to have had pneumonia. The suicide rate among this group of choreics is lower than that discussed above, largely because these choreics are selected for having been alive on April 1, 1940.

In reviewing these various measures of the progress of this disease, two main findings appear. Thus, in spite of the definitely chronic and progressive nature of Huntington's chorea, there is great individual variability in the onset and rate of progression of the disease as measured by ages at onset, institutionalization, and death. The other general finding is that there is no significant sex difference in these quantities, a result differing from some of those of the preceding section.

GENETICS OF HUNTINGTON'S CHOREA

The dominant inheritance of Huntington's chorea is apparent in all family studies and is accepted by all investigators, subject only to qualifications due to late onset and difficulties of diagnosis. Critical analysis of the segregation ratio among children of choreics, however, appears to have been done only by Sjögren (1935) and Panse (1942). Both found a satisfactory approach to a 1:1 ratio when a proper minimum age for classifying the children was adopted. In table 22 the proportion of choreic children in sibship data of the present study is given. Proportions for several different minimum ages were calculated because it is not obvious which age is best. One wishes to correct for both late onset and early death. As the table shows, satisfactory agreement is found for minimum ages 30 through 50 years. Family pedigrees show that the gene for Huntington's chorea is clearly autosomal in inheritance and the proportion of males among choreics in the sibships studied above agrees with this finding. For minimum ages 30, 40, 50, and 60 years, the proportions are 0.488, 0.476, 0.486, and 0.500, respectively, none of these differing significantly from 0.5. For this reason it is difficult to see how the significant deficiency of choreic males living in Michigan on April 1, 1940, found earlier, arose. Since other surveys have not found a deficiency it appears that this is only a chance finding, not likely to be repeated.

In table 23 the distribution is given of the number of known affected generations in kindreds containing a Michigan choreic on April 1, 1940. The data were usually

TABLE 22. PROPORTION OF CHOREICS AMONG CHILDREN OF CHOREICS. WHITE INDIVIDUALS IN SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940. SIBSHIPS WITH MORE THAN TWO DOUBTFULLY CHOREIC INDIVIDUALS ARE OMITTED. METHOD OF HALDANE (1938) IS USED AND COMPLETE ASCERTAINMENT IS ASSUMED.

Treatment of Dubious Choreics	Minimum Age o Classified Children			
	30 years	40 years	50 years	60 years
Considered choreic	0.529 ± 0.025	0.525 ± 0.027	0.486 ± 0.034	0.355 ± 0.049
Considered normal	0.484 ± 0.025	0.482*	0.450*	0.311*

* The standard error is very nearly that given directly above.

TABLE 23. NUMBER OF GENERATIONS AFFECTED WITH HUNTINGTON'S CHOREA. KINDREDS CONTAINING A WHITE MICHIGAN CHOREIC ON APRIL 1, 1940. LAY REPORTS OF CHOREA ACCEPTED.

No. of Choreic Individuals in Kindred on April 1, 1940	No. of Generations in Kindred Reported Affected							Total	Mean
	1	2	3	4	5	6	7		
1	21	23	16	3	5	1	0	69	2.29
2	2	9	10	10	3	0	1	35	3.20
3	1	2	3	2	2	1	0	11	3.45
4	0	0	1	1	2	0	0	4	4.25
5	0	0	0	2	1	0	0	3	4.33
6	0	1	0	0	1	0	0	2	3.50
Total	24	35	30	18	14	2	1	124	2.78

obtained from reasonably reliable informants instead of from medical sources. Those kindreds with only one or two affected generations known, almost always represent kindreds where no or little family investigation was possible or where one or more ancestors died relatively young. A critical discussion of kindreds with only one affected generation known and where both parents lived to age 60 or over, thus suggesting the occurrence of mutation, will be presented in the second part of this study. However, it is obvious from the table that new mutation accounts for only a small proportion of the observed cases of Huntington's chorea and that extensive pedigrees of affected individuals are common. Thus, 35 (out of 124) kindreds were studied in which, with reasonable reliability, four to seven affected generations were reported. These observations are in striking contrast to findings in the majority of well-studied rare dominant traits in man, such as achondroplasia (Mørch, 1941) or neurofibromatosis (Crowe, Schull, and Neel, 1955).

The heritability, i.e. degree of genetic determination, of (1) the age at onset of chorea, (2) age at death from chorea, and (3) interval between onset and death (measuring rate of progression) can be estimated from the sibship data. Analysis of variance, comparing variation within and between sibships, is the most convenient method of testing for such determinations and this analysis is presented in table 24. The most sensitive between-within sibships comparison uses the mean square for "between sibships, between kindreds"; i.e. it does not use the variation between

TABLE 24. ANALYSIS OF VARIANCE OF AGE OF ONSET, AGE AT DEATH, AND INTERVAL BETWEEN ONSET AND DEATH. SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940.

Variable	Source	S.S.	D.F.	M.S.	F	P
Age at onset	Between sibships, between kindreds	11,468.13	99	115.840	1.623	.05 > P > .01
	Between sibships, within kindreds	2,735.74	25	109.430	1.533	>.05
	Between sibships, total	14,203.87	124	114.547	1.604	.05 > P > .01
	Within sibships	5,710.22	80	71.378		
	Total	19,914.09	204	97.618		
Age at death	Between sibships, between kindreds	23,082.16	112	206.091	2.532	<.001
	Between sibships, within kindreds	5,147.09	30	171.570	2.107	<.01
	Between sibships, total	28,229.25	142	198.798	2.442	<.001
	Within sibships	8,710.84	107	81.410		
	Total	36,940.09	249	148.354		
(Age at death) minus (Age of onset)	Between sibships, between kindreds	5,171.36	85	60.840	1.196	>.05
	Between sibships, within kindreds	1,564.02	16	97.751	1.922	.05 > P > .01
	Between sibships, total	6,735.38	101	66.687	1.311	>.05
	Within sibships	2,085.43	41	50.864		
	Total	8,820.81	142	62.118		

sibships within the same kindred. A further comparison, testing whether there is a kindred resemblance apart from sibship resemblance, is possible if the ratio of mean squares of the two components of "between sibships" is obtained. None of these latter ratios is significant however. For onset and for death the variance between sibships is significantly larger than within sibships but this is not clearly the case for the onset-death interval. For this interval, however, the variance between sibships does exceed that within sibships. It seems reasonable to interpret the greater similarity among sibs in age at onset and at death as a consequence of their greater genetic similarity. Whether this increased similarity is due primarily to (a) the possession in common of similar "background" genes against which a gene for Huntington's chorea manifests itself, Huntington's chorea being due almost entirely, or exclusively, to one particular gene, or to (b) the possession in common of the same particular gene for Huntington's chorea, the same clinical appearance of Huntington's chorea being produced by any one of several different genes, at one or more loci, having frequencies which do not differ greatly, cannot be definitely decided from this analysis. Possibility (a) seems more likely, however, because the "between sibships, within kindreds" mean square exceeds the "within sibships" mean square for all three variables. A given "background" of two or more genes, present in one choreic, is more likely to be found in his sib than in a more distant relative, while the same allele producing chorea should be present throughout the kindred.

In other studies the similarity between sibs has more often been tested by finding the intra-class correlation coefficient and testing whether it differs significantly from zero. Fieller and Smith (1951) have given reasons for preferring the analysis of variance to the latter procedure. They also give formulas for deriving an improved estimate of the correlation coefficient from the components of variance. Using their procedure, the sib-sib coefficients for onset and for death are 0.28 and 0.47, respectively, and these are already known to be significantly different from zero. Bell (1934), using data from the literature, calculated a sib-sib correlation coefficient of 0.64. She discusses the possibility of bias due to utilizing incomplete histories in which sibs who may become choreic later are counted as normal. This bias would spuriously increase the correlation. Also, in the product-moment method, the large sibships are unduly weighted with respect to the smaller sibships (cf. Fieller and Smith, 1951), another source of bias. The sibships used in the present study were ascertained as of 1940 and followed until 1956, so that the first source of bias should be small, while deriving the estimate from the variances should minimize the second. It is therefore not too surprising that the two estimates differ appreciably. The higher correlation of age at death agrees with a general impression that in some kindreds there is marked similarity in the age at death from chorea, especially when at low ages. The correlation in age at death between normal sibs may also account for the higher value. Beeton and Pearson (1899) found a significant correlation, 0.26, between brothers in age at death.

The frequency in Michigan of individuals heterozygous for the gene determining Huntington's chorea, as distinguished from persons recognized as choreics, is of interest and can be approximately estimated. Since heterozygotes cannot be identified before onset, all methods of estimation must be indirect and each has certain

biases. In this situation the following approach seems as good as any. If H is the number of observed Huntington's choreics in an area at time t, P_x is the proportion of heterozygotes whose chorea is recognized by age x, N_x is the total number of individuals in the area at time t who are age x, and f is the frequency of heterozygotes in the general population in this area at time t, then

$$f = \frac{H}{\sum_x N_x P_x}$$

summing over all ages. We have determined H for the Lower Peninsula of Michigan in 1940 (203 individuals) and the N_x's for this area and time are known from the census of that year. P_x can be approximated from the observed age at onset curve, using the grand total distribution in table 15. This distribution, with mean 35.30 years and standard deviation 9.80 years, agrees closely with the corresponding normal distribution ($\chi^2_7 = 7.32$) so that values for P_x can be estimated from the normal curve. These values are slightly low because of the heterozygotes who are omitted from the onset data, due to failure to develop chorea by the time of the survey, either because of death or, if living, because the age at onset had not been reached. The segregation proportions show that this bias is small. Using the above-discussed values, the frequency of heterozygotes is estimated as 1.01×10^{-4} or one in 9,900 individuals. The gene frequency, of course, is half this value. The estimate of f is for the whole population and, because of mortality due to Huntington's chorea, the age-specific values will be somewhat higher for lower ages than high ones. Since the frequency of recognized choreics is estimated to be 4.12×10^{-5}, this means that only about 40 per cent of heterozygotes were identified as such. Because both numerator and denominator in the estimate are probably underestimates, the direction of probable bias of this estimate is unknown. The magnitude of the bias is also unknown but it seems unlikely to exceed about 10 per cent since the biases of numerator and denominator tend to cancel each other.

SUMMARY

In a study undertaken primarily to obtain data on natural selection and mutation in Huntington's chorea (to be analyzed in a following paper) the Lower Peninsula of the state of Michigan, U. S. A., was surveyed in 1954–1956 in an attempt to locate and investigate all individuals in this area, living or dead, ever institutionalized or not, who have developed this disease. A count of such persons as of April 1, 1940 yielded 203 individuals in 124 kindreds (groups of biologically related individuals). The population of this area in 1940 was 4,932,562, giving a frequency of choreics of 4.12×10^{-5} or about 1 in 24,000 individuals. The frequency of individuals heterozygous for the dominant gene for Huntington's chorea is estimated as 1.01×10^{-4}.

Certain demographic and social characteristics of choreics were compared with those of their normal sibs. When possible additional comparisons were made with the 1940 U. S Census. There were no marked differences between the non-choreic sibs and the population of the State of Michigan with respect to occupation and marital status. Choreics appeared to differ from their normal sibs and from the population of Michigan in a number of respects.

The dominant inheritance of the disease was confirmed. Data on the progress of the disease, as indicated by age at onset, institutionalization, and death, and the intervals between these ages, were obtained. Among sibs the ages at onset and the ages at death are significantly correlated, the correlation coefficients being 0.28 and 0.47. The sib-sib correlation for the rate of progress of the disease, as shown by the onset-death interval, is not significantly different from zero. Analysis of variance suggests that the sib-sib correlations are probably due to similar genetic backgrounds rather than to the possession in common of the same particular gene for Huntington's chorea, if several such genes, at one or more loci, do in fact exist.

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BOOK REVIEWS

Effect of Radiation on Human Heredity

Report of a study group convened by WHO together with papers presented by various members of the group. Published by World Health Organization, Geneva; distributed in the United States by Columbia University Press, New York. $4.00. 168 pp., paper bound.

In August 1956 just preceding the First International Congress of Human Genetics, The World Health Organization brought together in Copenhagen 20 scientists from several countries with interest and experience in problems of radiation biology, 12 of them geneticists, others concerned with statistics, radiation therapy or measurement or public health. Eleven of these presented statements of special aspects of the general problem of radiation effects, for discussion by the group. These eleven papers constitute the publication under review together with a brief report by the group.

The papers vary widely in content, length, and purpose. The longest paper is by H. J. Muller, University of Indiana, on "Damage from Point Mutations in Relation to Radiation Dose and Biological Conditions". This follows previous publications by the author in estimating spontaneous and induced mutation rates in Drosophila and the house mouse, and in discussing the problems involved in extrapolating from one species to another. "Damage" is estimated from the effects of a mutation when homozygous multiplied by its heterozygous effect. Actually this kind of extrapolation (from individual to population) involves a greater degree of uncertainty than in estimating mutation rates in one species from data obtained on another one. An estimate of the spontaneous lethal mutation rate in man is obtained from mortality of offspring of consanguineous as compared with nonconsanguineous marriages. This turns out to be 0.1 per gamete, that is, about one gamete in 10 is expected to contain a newly arisen deleterious mutation. This again depends upon assuming values for dominance of such mutations as borrowed from estimates for Drosophila. In respect to radiation, Muller points out the lack of experimental data on the relation of low dosages (below 25 r) to mutation rate, with consequent uncertainty whether (in this range) the relation between dose and mutation rate is linear, although he gives other reasons for thinking that it is. One misses throughout the paper a consideration of mutations, spontaneous or induced, as events which happen to ***populations***, yet it is the reaction of the population to new variants which enter it that gives them evolutionary significance. The stubborn facts that mutations which are disadvantageous or lethal when homozygous are still retained in populations and that population structures which make this possible have evolved must still be taken into account in any attempt to assess the future effects of mutations on populations. Fortunately this viewpoint is stressed in the brief statements of Bruce Wallace (Long Island Biological Laboratory) on "Some of the Problems Accompanying an Increase of Mutation Rates in Mendelian Populations", and of T. C. Carter (Atomic Energy Research Establishment, Harwell), on "Types of Mutation Produced at Known Gene Loci, and Possibility of Hitherto Unrecognized Mutations being Produced." A chief conclusion of the last named author is that "we have no mandate from experimental fact to extend to the whole human genome the theoretical treatment of the genetic hazard of radiations that we now apply with a fair measure of confidence to grossly deleterious gene mutations". Wallace contrasts the two concepts of the genetic structure of populations sharply and succinctly: the first based on superiority of homozygotes; the second upon superiority of heterozygotes.

In the first the deleterious effect of mutations on the population is a function of mutation rates; in the second the frequency of the constituent genes is primarily a function of selection and only secondarily a function of mutation rates. Although these are not mutually exclusive, the author's opinion is clearly that theoretical development of the second model holds greater promise for solving some of the problems which accompany increases of mutation rates in human populations.

The other long paper is that by R. M. Sievert (Karolinska Hospital, Stockholm) on "Exposure of Man to Ionizing Radiations, with Special Reference to Possible Genetic Hazards." This contains basic data, in tables and charts, on the distribution of ionizing radiations in the environment of human populations. Cosmic radiation, as known, is an important source only at very high altitudes. There it is high enough so that in computing risk figures for an individual one must take into account the fraction of his reproductive life that he spends in airplanes. Gamma radiation from rocks and from cement and brick buildings may provide as much as 3.2 roentgens dose per 30 years in concrete houses. After distant hydrogen bomb explosions, gamma radiation rises steeply. In this connection the author says "It does not yet seem possible to estimate the doses to human tissue due to fallout, nor their distribution in time, which are necessary data for judging the possible biological significance. Experience during the past year is, however, likely to raise doubts as to lack of biological importance of the tests of nuclear weapons, at any rate if they are continued on the present scale."

Shorter communications deal with special aspects: detection of induced mutations in offspring of irradiated parents (J. Lejeune, Paris); doses from diagnostic and therapeutic radiology (W. M. Court-Brown, Edinburgh); inbreeding levels in human populations (N. Freire-Maia, Parana); mutation rates in man (J. V. Neel, Ann Arbor; L. S. Penrose, London; A. C. Stevenson, Belfast); areas of high background radiation levels in India (A. R. Gopal-Ayengar, Bombay). The final paper is an interesting proposal by H. B. Newcombe, Atomic Energy of Canada, for a comprehensive and continuing survey of genetic trends in public health by vital statistics methods, based on a family register index from which degrees of relationship between spouses could be correlated with birth, death, and morbidity data.

The emphasis throughout the statements is on the future, reflecting a general recognition of the fact that most of the observations on which judgments of the effect of radiation on human heredity must rest, have still to be made. Consequently, the brief group report which precedes the statements might well have been entitled "What Ought to be Done". More research is of course the prime desideratum, but of what kinds? Nearly all kinds, according to the report, for although it does not recommend specific projects, it lists those areas in which there are serious gaps in our knowledge, and these cover nearly the whole range of human genetics. This may serve a useful purpose in making the medical profession and the public aware of our genetic unpreparedness for the atomic age; but some discrimination between more and less urgent problems would have been more helpful for those who have to set priorities.

The recommended strategy of advance along the whole broad front should be effectuated, according to the concluding section of the group report, by the establishment of institutions and university departments of human genetics. The bottleneck here, as pointed out, is lack of trained personnel, and this cannot be made good quickly. Our trouble is, of course, that in times of revolutionary change like the present, we are confronted with multiple emergencies with synergistic relations. Twelve years is a short interval between atom bomb and sputnik, and future intervals between major advances are likely to be shorter. Unpreparedness in human genetics is only one among many; we shall live hereafter with emergencies for which we are unprepared.

At the end of each such discussion of the future, we should ask ourselves the final question: what gives the problem discussed its emergency character? In many cases, as in this one, it is the danger of war. Perhaps our final conclusion should be to devote some fraction of our research effort, in all fields of thought, to the problems of human reconciliation which in our time have come to underlie all others.

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General Zoology

By MARY J. GUTHRIE AND JOHN M. ANDERSON. New York: John Wiley and Sons, Inc. 1957, pp. xv + 709 + 1 folding chart. $7.50.

GUTHRIE AND ANDERSON have thoroughly revised the old, successful textbook by Curtis and Guthrie ("Textbook of General Zoology"), and not only have maintained most of the good points of the older book, but also have added certain valuable new features. Notably, they have strengthened the handling of the physiological aspects of their subject, and they have attempted to correlate the usual cytological descriptions with recent discoveries using the electron microscope.

This revision of Curtis and Guthrie maintains the point of view and the basic organization of its predecessor. After a discussion of cells and their metabolism, three chapters are devoted to the chordates. These are followed by one chapter on heredity and variation, one on classification, and ten on the "invertebrate" phyla. Another chapter on the Chordata (and Hemichorda) is followed by a discussion of ecology and a chapter on evolution. The basic orientation throughout most of the book is the same as that of the first edition three decades ago, namely that morphology and taxonomy are the basic aspects of zoology. Descriptions are given of the structure of the usual series of animals: *Amoeba*, *Paramecium*, the hydra, *Dugesia*, *Lumbricus*, *Cambarus*, *Asterias*, *Rana*, etc., supplemented by brief descriptions of other related forms. Physiology is brought in, but only sketchily, and, so-to-speak, through the back door.

As in most zoology texts, there is no unifying concept other than anatomy and taxonomy, that ties the discussion together throughout the book. Evolution, which could serve well as such a unifying concept, is valiantly avoided as much as possible until the last chapter.

Evolution is a basic concept in biology, and the weakness of deferring any real discussion of it to the last chapter is indicated in Guthrie and Anderson by the dryness and lack of unity of the ten chapters on the invertebrate phyla. These chapters will become a chore for most students, for each is a dead account of anatomy and classification, in which the uninformed student will see no unifying principle, because none is offered.

Physiology, ecology, and heredity also could serve unifying roles if properly handled. But physiology is treated largely as if it were a subdivision of morphology. Ecology is discussed only in one chapter, near the end of the book. Heredity also is largely relegated to one chapter, with further acknowledgements of its importance in the final chapter on evolution. The discussion of ecology and heredity are very good summaries, considering the small amount of space devoted to them, but they are artificially isolated to a great extent from the general subject matter of the book.

It is the reviewer's opinion that most beginning zoology textbooks suffer, as does that of Guthrie and Anderson, from a "cut-and-dried" method of presenting the subject. Everything is presented as established fact, and the student seldom or never is taken to those regions where knowledge is tentative, and where our information is so scanty that there are substan-

tial differences of opinion among experts, or if such regions of knowledge are approached, only one of the possible and existing points of view is presented. Evolution is correctly described by Guthrie and Anderson as a fact, proven by adequate evidence, but only the prevailing viewpoint among biologists is described as acceptable when they discuss the concepts of how evolution came about (although on pg. 616 it is admitted that "One of the most challenging problems of the present day—one which has intrigued several generations of biologists—is that of determining the mechanisms or processes through which organic evolution has come about"). Would it not be more stimulating to the student to read, not only that most biologists believe that Lamarck's concept of the inheritance of acquired characteristics lacks validity, but also to learn that a group of investigators (among them Dürken, Just, Hovasse, Lindsey, Mani, and Martin) think they have valid evidence in support of a Neo-Lamarckian point of view?

Student interest may be anaesthetized when the authors speak of the origin long ago of living things from non-living, for they content themselves with saying: "We may simply reason as follows: life was not always present upon earth, and since life is now present, it must have originated at some time from non-living materials." Not a shred of the considerable evidence for such an origin is given; much less is there any discussion whatsoever of the stimulating differences of opinion concerning the evolutionary steps of such an origin. The coacervates of Oparin and Wald are not "too deep" to be understood by alert college students, nor are other hypotheses that have been presented in this field beyond their grasp.

In these fields, as well as in others not discussed in this review, the student who is presented the available evidence in an objective manner can draw his own conclusions, and thus add invaluably to his training as a scientist, or for the non-science major, to his understanding and appreciation of the scientific method, and of the fluid nature of scientific conclusions at the boundaries of knowledge.

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Enuresis: A Clinical and Genetic Study

By BERTIL HALLGREN. Copenhagen: E. Munksgaard, 1957, pp. xii + 159, 64 tables.

IN THIS NEW ADDITION to the outstanding Scandinavian series of monographs dealing with carefully conducted studies in clinical genetics, data are presented on 248 enuretic probands and their families. Clinically, a distinction is made between primary enuresis (never ceased to wet) and acquired enuresis (recurrence of wetting after a dry period of one year). The former category is assumed to include a "nuclear group" of enuretics in whom the basic disturbance seems to be genetically mediated, often in association with other signs of emotional disequilibrium.

Compared with girls, boys have a later age of cessation and a more frequent combination with encopresis (involuntary defecation). In some cases, the disorder is associated with retarded intellectual development. Of greater importance may be the observation that pyloric stenosis (radiologically unverified) is eight times as common in enuretic boys as it is in the general population. No evidence was obtained for an increased combination of enuresis with urinary tract disorders, premature birth, complicated delivery, or convulsive disease.

The symptomatology of a "problem child" as defined by the author was observed in 41 per cent of the probands and in 26 per cent of their enuretic sibs. In particular, such symptoms as behavior and speech disorders, emotional immaturity and general nervousness were

found significantly increased in the enuretic group. Excessive stress factors may often precipitate an "acquired" type of the disorder, and the children with both diurnal and nocturnal enuresis seem to be distinguished by an increased incidence of broken homes and psychiatric disorders in the mothers.

Comparative morbidity risk figures (enuresis) are presented for the sibs, parents and their sibs, and grandparents of the probands according to the various groupings of the sample, although ascertainment of the disorder in members of the older generations was admittedly inexact. Nevertheless, a significant increase in enuresis rates is revealed for the sibs and parents of enuretics. No definite statement is made regarding the usual mode of inheritance, but the author favors the theory of simple dominance with a low rate of penetrance, further reduced in the female.

The collection and analysis of the extensive clinical and genealogical data of this study represent a prodigious effort on the part of the author, especially since the investigation seems to have been conducted in accord with conservative standards of procedure. Unfortunately, the results are somewhat disappointing from a genetic standpoint as has happened quite frequently in studies where deviations from simple models of Mendelian inheritance are encountered. In the absence of an etiologically homogeneous and fully penetrant trait, the author's cautiously posited conclusions are necessarily expressed in terms of "likelihoods" and "suggested interpretations."

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Die Verteilung der ABO-Blutgruppen in der Schweitz

By S. ROSIN. Archiv der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene. Band XXXI (1956) Heft 1/2: 17–127.

ABO BLOOD GROUP FREQUENCIES are presented for 3,101 Swiss communities ranging in size from largest cities to smallest villages, also for 396 so-called "homogeneous groups"—clusters of neighboring communities empirically united until the composition of each is shown to be significantly distinct from that of its neighbors. The data were collected from 275,664 army conscripts and air raid wardens in 1940–1945, amounting to 6.8 per cent of the population of Switzerland. Since military conscription is obligatory for all men the data are distributed evenly throughout the entire country. They offer a rich and unique source for the study of blood group frequency distribution over a broad territory including a multitude of widely varying environments and a large number of biological-geographical isolates.

Since the subjects are grouped according to "community of origin" (a term which appears on all Swiss documents of identification, referring to the remote ancestors' locale) instead of present residence the samples represent a geographical distribution as of several generations ago. The geographical distribution as of 100 years ago is said to be twice as accurately represented by this method as by present residence.

The body of the data are presented in two tables, one for the 3,101 communities and the other for the 396 "homogeneous groups". The latter includes estimates of the three gene frequencies together with "D" (difference between preliminary estimates of p plus q plus r and 100 per cent, where p, q and r represent the frequencies of the three alleles *A*, *B* and *O*, respectively). The errors of army ABO determinations are reportedly about two per cent, which produce an error in gene frequency estimates of only about one per cent. The gene

frequencies are shown on two large maps of Switzerland, one for the communities having more than 17 subjects, 2,518 in number, the other for the 396 "homogeneous groups". The percentage composition of p, q, and r for each community and "homogeneous group" is represented by a color selected from an ingenious key of colors indicating the degree of departure of each of the three gene frequencies from the national average, which is estimated as 29.6 per cent p, 5.9 per cent q and 64.5 per cent r. On the map of communities each location is indicated by a circle of appropriate color, its size proportional to the population and its margin more heavily lined in case the difference between its pqr make-up and that of the nation is significant at the one per cent level.

The maps indicate that, ignoring local variations, Switzerland can be divided into four serological regions as follows: (a) northeast, high in *A*, low in *B*, low in *O*; (b) northern Alps, central and northwest, high in *A*; (c) west, a mosaic; (d) south and east Alps, high in *O*. Other studies have reported similarly high frequencies of *O* in the fourth region, greater than among the Sardinians, Basques or indeed any other population of Europe. All four regions cut across cantonal boundaries and do not correspond with the distribution of either language or religious grouping. No explanation is given for them. The cities are statistically indistinguishable from the national average, as expected.

The most striking result of the work is the high degree of heterogeneity throughout the country. Variation among the communities and "homogeneous groups" shows no isocline, progression, trend or transition in any gene frequency, such as have been described for other countries of Europe and for the continent as a whole. One wonders whether a gene frequency distribution study of other countries based on units as small as these communities might give similar results, particularly in areas which have had as little movement of peoples; also, whether a similar result might have been obtained in other long-settled areas a century or two ago, before the isolates had been quite so thoroughly broken up. The phenomenon is marked in the mountains but is still more striking in areas where no natural barriers exist, such as throughout the open rolling country of the entire north.

Some of the most extremely aberrant "homogeneous groups" are cited for special comment to illustrate their heterogeneity,—which is statistically significant even though the boundary of each group is drawn in the laboratory. Among them p varies from 19 per cent to 42 per cent, q from 2 per cent to 12 per cent and r from 51 per cent to 77 per cent. The author offers several theoretical explanations of these exceptionally deviating populations, including that of immigration of diverse ethnic groups (presuming a consequent relative failure to mix), genetic drift, and natural selection. While no conclusion is reached, the influence of selection varying from place to place under varying conditions of environment is said to be most unlikely.

The reviewer knows of no comparable source of material for investigating human variation and adaptation. Since the data on such environmental conditions as diet, disease, altitude and climate are well known, these factors can be rather definitively dismissed from consideration. Racial or physical traits, on the other hand, have not been carefully compared with these gene frequencies.

It is believed that the author is planning to repeat this tremendous compilation with data including Rh material now being collected by the Swiss Red Cross from all conscripts reporting currently for their first physical examination.

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BIOLOGIE DU NOIR. Matériaux et Recherches

By GEORGE A HEUSE. Brussels: Les Editions Problemes d'Afrique Centrale, 1957. xviii + 347 pp.

THIS LITTLE BOOK is a compilation of results, some of them due to the author, but most of them culled from the literature, of studies on Negroes in fields of serology and hematology, biochemistry, physiology, neurology and psycho-physiology, and ethnobiology. Three appendices describe technics, organization of the studies and subjects investigated, and a list of institutions where similar work is being carried out.

As the author himself admits, in the case of the majority of characteristics studied, comparison of Negroes with whites indicates no difference. In some cases where the author states there is a difference it can be seen on closer reading of the text that the difference is not statistically significant (the author presents mean values, or sometimes ranges, in his tables, but sometimes gives standard deviations in the text). Therefore, although this book might be a useful compendium for researchers interested in numerical results of tests on Negroes, it will have little value for the racial taxonomist.

An exception is of course provided by the blood group data, as it is well known that African blood group frequencies differ in a number of significant ways from those of other races. Some of the most important of these differences, however, such as the high frequency of Fy (a-b-) in Africans, were evidently unknown to the author. The blood group tables give frequencies of phenotypes only, without estimates of gene frequencies.

This may be no oversight. It would seem that the author does not believe much in genetic methods in physical anthropology, especially not in blood groups. He quotes with approval a remark by Schreider that their " . . . utilization systématique comme celle faite par BOYD, fait réapparaître 'un système classificatoire dont la tournure artificielle nous remène de deux siècles en arrière' ".

Unfortunately, there is evidence that the author is a bit behind the times himself, for he says " . . . nous avons parfois rapproché . . . les traits du Blanc lobotomisé et ceux du Noir normal. . . ." He also quotes with apparent approval the suggestion of a psychiatrist, said by him to be eminent, that one observes a "paresse des lobes frontaux . . . du Noir africain . . . ".

There is an author index and a subject index.

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LETTER TO THE EDITOR

Selective Survival in Dizygotic Twins

To The Editor
Dear Sir:

A large number of studies have been conducted recently with the goal of detecting selective factors which may operate to keep such polymorphic systems as the ABO antigens in balance. In the December, 1957, issue of this journal (vol. 9: 321–330) there appears an article entitled "Selective Survival in Dizygotic Twins in Relation to the ABO Blood Groups" by Richard H. Osborne and Francis V. DeGeorge which merits further consideration with regard to possible alternative explanations of the data. This article reports an increased concordance of dizygous twin pairs for the ABO antigens, citing as additional evidence studies by Schiff and v. Verschuer (*Z. Morph. Anthrop.* 32: 244, 1933) and by Gedda (*Studio dei Gemelli*, Rome: Edizioni Orizzonte Medico, 1951. p. 486).

Examination of the original reports of the latter two studies does not reveal an increased concordance for dizygous twins, however. In the study of Schiff and v. Verschuer, 156 of the 244 dizygous pairs examined were concordant, while 88 pairs were discordant. As explained in a footnote to the table in which these data were presented, the 88 discordant pairs are each counted under the two phenotypes found, giving a total of 176 discordant "pairs." The percentage concordance found is thus 47 per cent compared to a theoretical value of 45.6 per cent. If these figures are computed in the more usual way of counting each discordant pair only once, the observed concordance becomes 64 per cent with the theoretical value 62.7 per cent. The latter value was computed on theoretical bases and not arrived at empirically as stated by Osborne and DeGeorge. As Schiff and v. Verschuer point out, there is no difference in the frequency of concordance between dizygous twins compared to sibling pairs.

Gedda's material has been published only in review form and consists of 39 sets of dizygous twins of which 64 per cent (25 sets) were concordant in the ABO system. Of the 93 sib pair controls, 58 per cent were concordant. The difference between 58 and 64 per cent of 39 pairs is only 2 pairs, however, hardly enough to conclude that the two groups differ.

The data presented by Osborne and DeGeorge were obtained from three groups of dizygous twin pairs: 60 white adult pairs (18–55 years), 14 white juvenile pairs (4–8 years), and 20 Negro juvenile pairs (4–8 years). In the adult series, 70 per cent of the pairs were concordant, a value which does not differ significantly from expectation (the authors found $P = .20$). This series can hardly be used then to support an increased concordance among dizygous pairs. There remain the two juvenile series of which 13 of the 14 white pairs are concordant and 18 of the 20 Negroes ($P = .03$ and .01 respectively). In evaluating the significance of these results, the authors have ignored the fact that in the juvenile samples, and particularly in the Negroes, the ABO phenotype frequencies are hardly normal. In the Negroes, the values for A, B, AB, and O are 17.5, 7.5, 0.0, and 75 per cent respectively. These result in gene frequencies of $p = .092$, $q = .039$, and $r = .866$, which are appreciably different from the general population figures used for computing the expected concordance ($p = .198$, $q = .142$, $r = .660$). If one computes the expected concordance on the basis of the actual gene frequencies in the sample, thus assuming that the gene frequencies of the parents were unusual for some reason, the expected concordance becomes 78.2 per cent rather than 65.1 per cent. This leaves an excess of only two twin pairs over the expected value, with a non-significant χ^2 of 1.63. The remaining group of 14 juvenile whites continues to show an excess of concordance significant at the 5 per cent level if the probabilities are computed on the basis of actual gene frequencies in this group.

In our own laboratory we have had occasion to ascertain the ABO types of 37 pairs of twins classified as dizygous on the basis of differences in blood types and other generally accepted traits. The A group was further subdivided into A_1 and A_2. Of the 37 sets, 21 were concordant in the ABO system compared to an expected value of 23, again failing to show any suggestion of excess concordance.

The four series of adult twins, all of which fail to show significant deviation from expectation,

support the idea that any excess of concordance in dizygous twins must be at most very small. The juvenile series reported by Osborne and DeGeorge is more difficult to interpret. If one assumes an unusual distribution of genotypes in the parents of these twins, then what is the reason for this unusual distribution? One possibility which suggests itself is a differential tendency for twinning among the various blood types. Osborne and DeGeorge also mention the possibility of differential survival among twins of various blood types. These possibilities must be considered, however, against the fact that in all the adult series, including the extensive one of Schiff and v. Verschuer, the genotype frequencies match those of the general population very closely. Thus if a difference exists at birth or shortly after, it must be exactly counterbalanced by differential survival before adulthood. With respect to the possibility of immunological incompatibility, it may also be pointed out that fetuses and newborns are noted for their low capacity to form antibodies. Even if the formation of antibodies by the mother is taken into consideration, only 19 per cent of mother/single child combinations are potentially incompatible, and of these only a few per cent are believed to give rise to inviable fetuses. (See, for example, T. E. Reed, this journal, 8: 257, 1956.)

It seems unlikely then that the explanations advanced by Osborne and DeGeorge could account for the unusual distributions observed in their juvenile series. Indeed, the demonstration of selection of this type in twins will require much more data than have been collected by the various investigators.

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